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DESCRIPTION

FIELD OF INVENTION

[0001] The present invention relates to a humanised antibody which binds to serum amyloid P component (SAP), polynucleotides encoding such an antibody, pharmaceutical compositions comprising said antibody and methods of manufacture. The present invention also concerns the use of such an antibody in the treatment or prophylaxis of diseases associated with amyloid deposition including systemic amyloidosis, local amyloidosis, Alzheimer's disease, and type 2 diabetes.

BACKGROUND OF THE INVENTION

[0002] Amyloidosis is a serious and usually fatal disease caused by the extracellular accumulation in the tissues of abnormal insoluble protein fibres known as amyloid fibrils. These are derived from more than 20 different proteins in different forms of the disease but all amyloid fibrils share a common cross- β core structure and all are derived by misfolding of normally soluble precursor proteins (Pepys, M.B. (2006) *Annu. Rev. Med.*, 57: 223-241). A normal non-fibrillar plasma protein, serum amyloid P component (SAP), is also always present in amyloid deposits by virtue of its avid specific calcium dependent binding to all types of amyloid fibrils (Pepys et al. (1979) *Clin. Exp. Immunol.*, 38: 284-293; Pepys et al. (1997) *Amyloid: Int. J. Exp. Clin. Invest.*, 4: 274-295).

[0003] Human SAP is a constitutive protein in the plasma, at a concentration of around 20-40 mg/l (Nelson et al. (1991) *Clin. Chim. Acta*, 200:191-200) and with a total of about 50-100 mg of SAP in the combined plasma and extravascular compartments both of normal individuals and patients with diseases other than amyloidosis (Hawkins et al. (1990) *J. Clin. Invest.*, 86: 1862-1869). In patients with amyloidosis, SAP is also specifically concentrated in the amyloid deposits and in an individual with extensive systemic amyloidosis there may be as much as 20,000 mg of SAP in the amyloid (Pepys et al. (1994) *PNAS*, 91: 5602-5606), reversibly bound to the fibrils and in equilibrium with the fluid phase SAP pool. The normal physiological function of circulating SAP is poorly understood, but animal experiments and *in vitro* studies suggest a role in host defence (Noursadeghi et al. (2000) *PNAS*, 97: 14584-14589)). SAP is also a normal tissue matrix constituent associated with elastic fibres and the glomerular basement membrane although its function there is not known.

[0004] In amyloidosis, the extracellular amyloid deposits cause disease by progressive accumulation until they damage the structure and thus the function of whatever tissue they occupy (Pepys, M.B. (2006) *Annu. Rev. Med.*, 57: 223-241). There is very rarely any inflammatory or 'foreign body' response to amyloid deposition, either seen locally in the tissues or suggested by systemic markers of inflammation. Systemic amyloidosis can involve any organ, is usually fatal and causes ~1 per thousand deaths in developed countries. Localised amyloid, confined to a single anatomical location or tissue type, can also be very serious, for example cerebral amyloid angiopathy is an important cause of haemorrhagic stroke. The clinical presentations of amyloidosis are extremely diverse and the diagnosis is rarely made before significant organ damage is present. Over 20 different amyloid fibril proteins are responsible for different forms of amyloidosis, but treatments that substantially reduce the abundance of the respective amyloid fibril precursor protein do halt amyloid accumulation and the deposits may regress. Unfortunately effective measures are not always available and, when they do exist, are toxic or hazardous and slow to act (Pepys, M.B. (2006) *Annu. Rev. Med.*, 57: 223-241). There is therefore a major unmet medical need for therapy which safely promotes the clearance of established amyloid deposits. Furthermore, there are other conditions in which amyloid deposits are always present, most importantly Alzheimer's disease (AD) and type 2 diabetes mellitus, in which the contribution of amyloid deposition to the pathogenesis of disease, specifically loss of cognitive and pancreatic islet function, respectively, is not known (Pepys, M.B. (2006) *Annu. Rev. Med.*, 57: 223-241). However, amyloid deposits anywhere else in the body are demonstrably pathogenic and it is likely that the cerebral deposits of AD and the islet amyloid deposits of type 2 diabetes are also harmful. Since treatment which clears amyloid deposits in systemic amyloidosis will certainly be therapeutic (Pepys, M.B. (2006) *Annu. Rev. Med.*, 57: 223-241), removal of the amyloid deposits in AD and type 2 diabetes should also be clinically beneficial.

[0005] Binding of SAP stabilises amyloid fibrils, protects them from proteolysis *in vitro* (Tennent et al., (1995) *PNAS*, 92: 4299-4303), can enhance amyloid fibrillogenesis *in vitro* (Myers et al., (2006), *Biochemistry*, 45: 2311-2321) and contributes to pathogenesis of systemic amyloidosis *in vivo* (Botto et al., (1997) *Nature Med.*, 3: 855-859). Coupled with its universal presence in all amyloid deposits, these properties of SAP make it an attractive therapeutic target.

[0006] European patent application EP 0915088 discloses D-proline derivative compounds that are competitive inhibitors of

binding of SAP to amyloid fibrils, as well as methods for their manufacture. A preferred compound disclosed in EP 0915088 is (R)-1-[6-[(R)-2-Carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC).

[0007] International patent application WO 03/051836 discloses prodrugs for D-proline derivative compounds.

[0008] International patent application WO 2004/099173 discloses glycerol cyclic pyruvate derivatives that are competitive inhibitors of binding of SAP to amyloid fibrils.

[0009] International patent application WO 04/059318 describes methods which are asserted to enhance fibrocyte formation which comprise the provision of compositions which bind SAP. Such compositions include anti-SAP antibodies and CPHPC. WO 04/059318 does not disclose the treatment of disease associated with amyloid deposition. Furthermore, there is compelling clinical and *in vivo* evidence that neither SAP nor its depletion have any effect on fibrosis in humans (Tennent et al., (2007) Arthritis Rheum., 56: 2013-2017; Pepys, M.B., Tennent, G.A. and Denton, C.P. (2007) Reply to Letter from Pilling, D., Buckley, C.D., Salmon, M. and Gomer, R.G., Serum amyloid P and fibrosis in systemic sclerosis: comment on the article by Tennent et al. Arthritis Rheum., 56:4229-4230).

[0010] The bis-D-proline compound, CPHPC, disclosed in the patents listed above, is bound with high affinity by human SAP and was intended as a drug to remove SAP from amyloid deposits *in vivo* and thereby facilitate their clearance. Binding of CPHPC by SAP triggers rapid clearance of the complex by the liver, depletes almost all circulating SAP for as long as the drug is administered, and removes much but not all amyloid bound SAP (Pepys et al., (2002) Nature, 417: 254-259). In initial clinical studies (Gillmore et al., (2010) Brit. J. Haematol., doi:10.1111/j.1365-2141.2009.08036.x), administration of CPHPC seemed to arrest amyloid accumulation but it did not produce amyloid regression and since CPHPC does not completely remove all SAP from amyloid deposits, another approach is needed.

[0011] International patent application WO 2009/000926 discloses the use of compounds which deplete SAP from the circulation, such as D-proline derivatives, in particular CPHPC, in combination with an antibody specific for SAP for the treatment or prophylaxis of amyloidosis.

[0012] Related International patent application PCT/EP2008/011135 concerns various mouse monoclonal antibodies which may be used in combination with compounds which deplete SAP from the circulation, such as D-proline derivatives, in particular CPHPC, for the treatment or prophylaxis of amyloidosis.

[0013] Accordingly, there is a need in the art for antibodies, particularly humanised or human antibodies, which specifically target SAP and provide improved therapeutic efficacy in patients, particularly human patients, with diseases associated with amyloid deposition in order to preserve organ function and prolong life.

SUMMARY OF THE INVENTION

[0014] The present invention provides, in a first aspect, an antibody which specifically binds to SAP and comprises a heavy chain variable region of SEQ ID NO:28; and a light chain variable region of SEQ ID NO:35 and wherein the antibody comprises a human IgG1 or IgG3 human constant domain.

[0015] In a second aspect of the invention, there is provided humanised antibody which specifically binds to SAP and comprises a heavy chain of SEQ ID NO:62; and a light chain of SEQ ID NO:64.

[0016] The present invention also provides a nucleic acid molecule encoding a humanised antibody of the invention, expression vectors comprising the same, and host cells capable of producing antibodies of the invention.

[0017] In a further aspect of the invention a pharmaceutical composition comprising an antibody as defined herein is provided. The present invention also provides methods of preventing and/or treating a subject susceptible to or afflicted with a disease associated with amyloid deposition, which method comprises the step of administering a prophylactically or therapeutically effective amount of an antibody to said subject. The use of an antibody as defined herein for preventing and/or treating a subject susceptible to or afflicted with a disease associated with amyloid deposition is provided. The use of an antibody as defined herein for the manufacture of a medicament for preventing and/or treating a subject susceptible to or afflicted with a disease associated with amyloid deposition is also provided.

BRIEF DESCRIPTION OF THE FIGURES

[0018]

Figure 1 shows the binding curves for murine antibodies SAP-E and SAP-K at a 1 µg/mL coating concentration of human SAP.

Figure 2 shows the binding curves for murine antibodies SAP-E and SAP-K at a 5 µg/mL coating concentration of human SAP.

Figure 3 shows the binding curves for chimeric antibodies cSAP-E and cSAP-K. The profile of the curves for the chimeric antibodies is the same as that of the equivalent hybridomas.

Figure 4 shows the binding curves for SAP-K H0L0, SAP-K H1L0, SAP-K H2L0 and SAP-K H3L0 compared to the SAP-K chimera and the SAP-E H1L1 compared to the SAP-E chimera. An irrelevant human IgG1 kappa antibody was also tested as a negative control.

Figure 5 shows purified SAP-K and SAP-E murine monoclonal antibodies in a competition ELISA with the SAP-E chimera.

Figure 6 shows purified SAP-K and SAP-E murine monoclonal antibodies in a competition ELISA with the SAP-K chimera.

Figure 7 shows an immunoradiometric assay for binding of monoclonal mouse antibodies SAP-E and SAP-K to human SAP captured by immobilised sheep polyclonal anti-human SAP antibody.

Figure 8 shows epitope mapping for monoclonal anti-human SAP antibody SAP-E.

Figure 9 shows the location of the epitopes on human SAP recognised by SAP-K (A, highlighted in black) and SAP-E (B, shown in white).

Figure 10 shows C3 activation by humanised monoclonal anti-human SAP antibodies in whole human serum.

Figure 11 shows C3 activation by low dose humanised monoclonal anti-human SAP antibodies in whole human serum.

Figure 12 shows C3 activation by humanised monoclonal anti-human SAP antibodies in whole mouse serum supplemented with pure human SAP.

DETAILED DESCRIPTION OF THE INVENTION

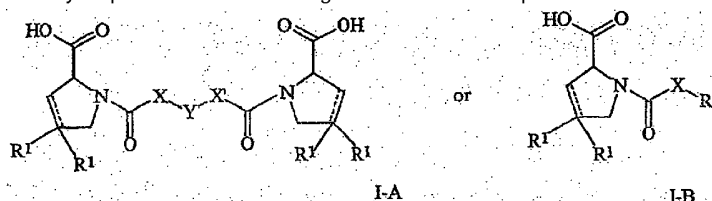
[0019] The present invention provides a humanised antibody which binds to serum amyloid P component (SAP), for example human SAP, as its specific antigen (i.e. a SAP binding protein). In therapeutic applications of the invention, the antibody activates the body's potent mechanisms for clearance of abnormal debris from tissues. The antibody may be a monoclonal antibody. An antibody of the invention is not a murine antibody.

[0020] "Serum amyloid P component" or "SAP" refers to a homopentameric plasma glycoprotein of the pentraxin family. Each molecule is composed of 5 identical protomers, each with a flattened β-jelly roll fold and single alpha helix, non-covalently associated in a disc-like ring with cyclic pentameric symmetry (Hutchinson et al., (2000) Mol. Med., 6: 482-493); Pepys et al., (2002) Nature, 417: 254-259). The term "SAP" as used herein also includes the individual subunit encoded by the human gene APCS (chromosome: 1; Location: 1q21-q23) or homologous genes in other organisms, for example the human SAP polypeptide subunit having the sequence as set forth in SEQ ID NO:43 as well as the native pentameric form of SAP, and any fragments and variants of SAP that retain the biological activity of binding to amyloid fibrils *in vivo*.

[0021] The antibody of the invention can bind to any one or any combination of the above described different forms of SAP. In a particular embodiment, the antibody of the invention binds human SAP. The antibody of the invention can bind to SAP when the SAP is bound to amyloid fibrils of any type and in any extracellular location within the body. The antibody of the invention may also bind to native unbound SAP.

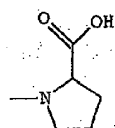
[0022] An essential aspect of utilising antibodies of the invention in therapeutic methods is that the concentration of SAP in the circulation must be reduced by at least 90% below its normal value before administration of the antibody. Specifically, this can be achieved by compounds that decrease the amount of circulating SAP and, in particular, compounds that result in the depletion of circulating SAP, defined here as "SAP depleting compounds". Such compounds are ligands bound by SAP and are competitive

inhibitors of the binding of SAP to amyloid fibrils, such as D-proline derivatives and glycerol cyclic pyruvate derivatives. D-proline derivatives are disclosed in EP 0915088, which is incorporated herein by reference in its entirety, and the term "D-proline derivatives" includes prodrugs, such as those disclosed in WO 03/051836, which is also incorporated herein by reference in its entirety. D-prolines of the following formula are contemplated:



wherein

R is



and the group

R¹ is hydrogen or halogen; and

X is $-(CH_2)_n-$; $-CH(R^2)(CH_2)_n-$; $-CH_2O(CH_2)_n-$; $-CH_2NH-$; $-C(R^2)=CH-$; $-CH_2CH(OH)-$; or thiazol-2,5-diyl; $-O-$;

Y is -S-S-; -(CH₂)_n-; -O-; -NH-; -N(R²)-; -CH=CH-; -NHC(O)NH-;

$$\text{N(R}^2\text{)C(O)N(R}^2\text{)-; -N[CH}_2\text{C}_6\text{H}_3\text{(OCH}_3\text{)}_2\text{]-; -N(CH}_2\text{C}_6\text{H}_5\text{)-;}$$

-N(CH₂C₆H₅)C(O)N(CH₂C₆H₅)-; -N(alkoxyalkyl)-;

N(cycloalkyl-methyl)-; 2,6-pyridyl; 2,5-furanyl; 2,5-thienyl; 1,2-cyclohexyl; 1,3-cyclohexyl; 1,4-cyclohexyl; 1,2-naphthyl; 1,4-naphthyl; 1,5-naphthyl; 1,6-naphthyl; or 1,2-phenylene, 1,3-phenylene and 1,4-phenylene, wherein the phenylene groups are optionally substituted by 1-4 substituents, selected from halogen, lower alkyl, lower alkoxy, hydroxyl, carboxy, -COO-lower alkyl, nitrilo, 5-tetrazol, (2-carboxylic acid pyrrolidin-1-yl)-2-oxo-ethoxy, N-hydroxycarbamimiodyl, 5-oxo[1,2,4oxadiazolyl, 2-oxo [1,2,3,5] oxathiadiazolyl, 5-thioxo[1,2,4]oxadiazolyl and 5-tert-butylsulfanyl-[1,2,4]oxadiazolyl;

X is $-(CH_2)_n-$; $-(CH_2)_nCH(R_2)-$; $-(CH_2)_nOCH_2-$; $-NHCH_2-$;

-CH=C(R²)-; CH(OH)CH₂; or thiazol-2,5-diyl; -O-;

R² is lower alkyl, lower alkoxy or benzyl,

n is 0-3 and wherein

alkyl or lower alkyl is C₁₋₆ alkyl; alkoxy or lower alkoxy is C₁₋₆ alkoxy; cycloalkyl is C₃₋₆ cycloalkyl; halogen is F, Cl or Br; and the location where the dotted line appears in the formula is either a single or double bond;

or a pharmaceutically acceptable salt or mono- or diester thereof.

[0023] D-prolines of formula I-A above can be written as Ligand - linker - Ligand, wherein the X-Y-X' moiety of formal I-A forms the linker. The linker (X-Y-X') can be from 4 to 20 linear carbon atoms in length, including from 4-15 linear carbon atoms, 5-10 linear carbon atoms, and 6-8 linear carbon atoms in length. The linker can be a straight or branched chain, or can optionally form one or more ring structures, with the proviso that at least 4 linear or straight-chain carbon atoms are present in the linker. At least one of the linear or straight-chain C atoms can be optionally substituted by at least one hetero atom selected from N, O, or S, advantageously O or S, advantageously O.

[0024] Thus, an "optionally substituted linker" can have one or more substitutions that lead to branching and/or one or more substitutions of carbon atom(s) of the linear or straight chain carbon atoms of the linker, e.g. the linker can be an ether or a substituted ether.

[0025] (R)-1-[6-[(R)-2-Carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC) is a specific D-proline contemplated by the invention. In a particular embodiment, CPHPC is to be administered to a human patient.

[0026] Glycerol cyclic pyruvate derivatives are disclosed in WO 2004/099173, which is incorporated herein by reference in its

entirety.

[0027] The term "antibody" is used herein in the broadest sense to refer to molecules with an immunoglobulin-like domain and includes monoclonal, recombinant, polyclonal, chimeric, humanised, bispecific and heteroconjugate antibodies; a single variable domain, a domain antibody, antigen binding fragments, immunologically effective fragments, single chain Fv, diabodies, Tandabs™, etc. (for a summary of alternative "antibody" formats see Holliger and Hudson, Nature Biotechnology, 2005, Vol 23, No. 9, 1126-1136).

[0028] The term "specifically binds" as used throughout the present specification in relation to antigen binding proteins means that the antibody binds to SAP with no or insignificant binding to any other proteins, including closely related molecules such as C-reactive protein (CRP) which, in humans, shares 55% of strict residue for residue amino acid sequence homology and has essentially the same protein fold.

[0029] The equilibrium dissociation constant (KD) of the antibody-SAP interaction may be 1 mM or less, 100 nM or less, 10 nM or less, 2 nM or less or 1 nM or less. Alternatively the KD may be between 5 and 10 nM; or between 1 and 2 nM. The KD may be between 1 pM and 500 pM; or between 500 pM and 1 nM.

[0030] The binding affinity may be measured by BIAcore™, for example by antigen capture with SAP coupled onto a carboxymethyldextran chip by primary amine coupling and antibody capture onto this surface. Alternatively, the binding affinity can be measured by BIAcore™ by binding of anti-SAP antibodies to human SAP captured by O-phosphoethanolamine immobilised on a CM5 chip. The BIAcore™ methods described in Example 8 may be used to measure binding affinity.

[0031] The dissociation rate constant (kd) may be $1 \times 10^{-3} \text{ s}^{-1}$ or less, $1 \times 10^{-4} \text{ s}^{-1}$ or less, or $1 \times 10^{-5} \text{ s}^{-1}$ or less. The kd may be between $1 \times 10^{-5} \text{ s}^{-1}$ and $1 \times 10^{-4} \text{ s}^{-1}$; or between $1 \times 10^{-4} \text{ s}^{-1}$ and $1 \times 10^{-3} \text{ s}^{-1}$. A small kd may result in a slow dissociation of the antibody-ligand complex and improved clearance of complexes of SAP bound to amyloid.

[0032] It will be apparent to those skilled in the art that the term "derived" is intended to define not only the source in the sense of it being the physical origin for the material but also to define material which is structurally identical to the material but which does not originate from the reference source. Thus "residues found in the donor antibody" need not necessarily have been purified from the donor antibody.

[0033] By "isolated" it is intended that the molecule, such as an antigen binding protein, is removed from the environment in which it may be found in nature. For example, the molecule may be purified away from substances with which it would normally exist in nature. For example, the mass of the molecule in a sample may be 95% of the total mass.

[0034] A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

[0035] A "humanised antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one or more human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al. Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al. Bio/Technology, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT® database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody. The prior art describes several ways of producing such humanised antibodies - see for example EP-A-0239400 and EP-A-054951.

[0036] The term "donor antibody" refers to an antibody which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments or analogs thereof to a first immunoglobulin partner. The donor therefore provides the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralising activity characteristic of the donor antibody.

[0037] The term "acceptor antibody" refers to an antibody which is heterologous to the donor antibody, which contributes all (or any portion) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. A human antibody may be the acceptor antibody.

[0038] The term "human antibody" refers to an antibody derived from human immunoglobulin gene sequences. These fully human antibodies provide an alternative to re-engineered, or de-immunized, rodent monoclonal antibodies (e.g. humanised antibodies) as a source of low immunogenicity therapeutic antibodies and they are normally generated using either phage display or transgenic mouse platforms. In an embodiment, an antibody of the invention is a human antibody.

[0039] The terms "VH" and "VL" are used herein to refer to the heavy chain variable region and light chain variable region respectively of an antigen binding protein.

[0040] "CDRs" are defined as the complementarity determining region amino acid sequences of an antigen binding protein. These are the hypervariable regions of immunoglobulin heavy and light chains. There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, all three light chain CDRs, all heavy and light chain CDRs, or at least two CDRs.

[0041] Throughout this specification, amino acid residues in variable domain sequences and full length antibody sequences are numbered according to the Kabat numbering convention. Similarly, the terms "CDR", "CDRL1", "CDRL2", "CDRL3", "CDRH1", "CDRH2", "CDRH3" used in the Examples follow the Kabat numbering convention. For further information, see Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987).

[0042] However, although we use the Kabat numbering convention for amino acid residues in variable domain sequences and full length antibody sequences throughout this specification, it will be apparent to those skilled in the art that there are alternative numbering conventions for amino acid residues in variable domain sequences and full length antibody sequences. There are also alternative numbering conventions for CDR sequences, for example those set out in Chothia et al. (1989) Nature 342: 877-883. The structure and protein folding of the antibody may mean that other residues are considered part of the CDR sequence and would be understood to be so by a skilled person.

[0043] Other numbering conventions for CDR sequences available to a skilled person include "AbM" (University of Bath) and "contact" (University College London) methods. The minimum overlapping region using at least two of the Kabat, Chothia, AbM and contact methods can be determined to provide the "minimum binding unit". The minimum binding unit may be a sub-portion of a CDR.

[0044] Table 1 below represents one definition using each numbering convention for each CDR or binding unit. The Kabat numbering scheme is used in Table 1 to number the variable domain amino acid sequence. It should be noted that some of the CDR definitions may vary depending on the individual publication used.

Table 1

	Kabat CDR	Chothia CDR	AbM CDR	Contact CDR	Minimum binding unit
H1	31-35/35A/35B	26-32/33/34	26-35/35A/35B	30-35/35A/35B	31-32
H2	50-65	52-56	50-58	47-58	52-56
H3	95-102	95-102	95-102	93-101	95-101
L1	24-34	24-34	24-34	30-36	30-34
L2	50-56	50-56	50-56	46-55	50-55
L3	89-97	89-97	89-97	89-96	89-96

[0045] As used herein, the term "antigen binding site" refers to a site on an antigen binding protein which is capable of specifically binding to an antigen. This may be a single domain (for example, an epitope-binding domain), or single-chain Fv (ScFv) domains or it may be paired VH/VL domains as can be found on a standard antibody.

[0046] The term "epitope" as used herein refers to that portion of the antigen that makes contact with a particular binding domain of the antigen binding protein. An epitope may be linear, comprising an essentially linear amino acid sequence from the antigen. Alternatively, an epitope may be conformational or discontinuous. For example, a conformational epitope comprises amino acid residues which require an element of structural constraint. In the case of a conformational epitope, although the residues may be

from different regions of the peptide chain, they may be in close proximity in the three dimensional structure of the antigen. In the case of multimeric antigens, such as SAP, a conformational epitope may include residues from different peptide chains that may be in close proximity in the three dimensional structure of the antigen. Such structurally neighbouring residues can be determined through computer modelling programs or via three-dimensional structures obtained through methods known in the art, such as X-ray crystallography.

[0047] A discontinuous epitope comprises amino acid residues that are separated by other sequences, i.e. not in a continuous sequence in the antigen's primary sequence. In the context of the antigen's tertiary and quaternary structure, the residues of a discontinuous epitope are near enough to each other to be bound by an antigen binding protein.

[0048] In an embodiment, an antibody of the invention binds to an epitope within residues 140-158 of human SAP.

[0049] For nucleotide and amino acid sequences, the term "identical" or "sequence identity" indicates the degree of identity between two nucleic acid or two amino acid sequences when optimally aligned and compared with appropriate insertions or deletions.

[0050] The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions multiplied by 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm, as described below.

[0051] The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (Comput. Appl. Biosci., 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (J. Mol. Biol. 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0052] By way of example, a polynucleotide sequence may be identical to a reference polynucleotide sequence as described herein (see for example SEQ ID NO:8, 10, 18, 20, 45-48, 51-61, 63, 65-73), that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, such as at least 50, 60, 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identical. Such alterations are selected from at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in the reference polynucleotide sequence as described herein (see for example SEQ ID NO:8, 10, 18, 20, 45-48, 51-61, 63, 65-73), by the numerical percent of the respective percent identity (divided by 100) and subtracting that product from said total number of nucleotides in the reference polynucleotide sequence as described herein (see for example SEQ ID NO:8, 10, 18, 20, 45-48, 51-61, 63, 65-73), or:

$$n_n \leq x_n - (x_n \cdot y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in the reference polynucleotide sequence as described herein (see for example SEQ ID NO:8, 10, 18, 20, 45-48, 51-61, 63, 65-73), and y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.75 for 75%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.98 for 98%, 0.99 for 99% or 1.00 for 100%, \cdot is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n .

[0053] Similarly, a polypeptide sequence may be identical to a polypeptide reference sequence as described herein (see for example SEQ ID NO:1-7, 9, 11-17, 19, 21-24, 27-31, 34-42, 62, 64, 74), that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%, such as at least 50, 60, 70, 75, 80, 85, 90, 95, 96, 97, 98, or 99% identical. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined

by multiplying the total number of amino acids in the polypeptide sequence encoded by the polypeptide reference sequence as described herein (see for example SEQ ID NO:1-7, 9, 11-17, 19, 21-24, 27-31, 34-42, 62, 64, 74) by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in the polypeptide reference sequence as described herein (see for example SEQ ID NO:1-7, 9, 11-17, 19, 21-24, 27-31, 34-42, 62, 64, 74), or:

$$n_a \leq x_a - (x_a \cdot y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in the reference polypeptide sequence as described herein (see for example SEQ ID NO:1-7, 9, 11-17, 19, 21-24, 27-31, 34-42, 62, 64, 74), and y is, 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.75 for 75%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.98 for 98%, 0.99 for 99%, or 1.00 for 100%, \cdot is the symbol for the multiplication operator, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

[0054] The % identity may be determined across the length of the sequence.

[0055] The terms "peptide", "polypeptide" and "protein" each refers to a molecule comprising two or more amino acid residues. A peptide may be monomeric or polymeric.

[0056] It is well recognised in the art that certain amino acid substitutions are regarded as being "conservative". Amino acids are divided into groups based on common side-chain properties and substitutions within groups that maintain all or substantially all of the binding affinity of the antibody are regarded as conservative substitutions, see Table 2 below:

Table 2

Side chain	Members
Hydrophobic	Met, Ala, Val, Leu, Ile
Neutral hydrophilic	Cys, Ser, Thr
Acidic	Asp, Glu
Basic	Asn, Gln, His, Lys, Arg
Residues that influence chain orientation	Gly, Pro
Aromatic	Trp, Tyr, Phe

[0057] The antibody may compete for binding to SAP with a reference antibody comprising a heavy chain variable region sequence of SEQ ID NO: 7, and a light chain variable region sequence of SEQ ID NO: 9. Alternatively, the antibody may compete for binding to SAP with a reference antibody comprising a heavy chain variable region sequence of SEQ ID NO: 17, and a light chain variable region sequence of SEQ ID NO: 19.

[0058] Competition between the antibody and the reference antibody may be determined by competition ELISA, FMAT or BIAcore. A competing antibody may bind to the same epitope, an overlapping epitope, or an epitope in close proximity of the epitope to which the reference antibody binds.

[0059] The corresponding CDRs can be defined by reference to Kabat (1987), Chothia (1989), AbM or contact methods, or a combination of these methods. One definition of each of the methods can be found at Table 1 and can be applied to the reference heavy chain variable domain SEQ ID NO:7 and the reference light chain variable domain SEQ ID NO:9 to determine the corresponding CDR.

[0060] The invention also provides an antibody which specifically binds to SAP and comprises a heavy chain variable region of SEQ ID NO:28 and a light chain variable region of SEQ ID NO:35.

[0061] The antibody comprises the following heavy chain and light chain variable region combination: H1L1 (SEQ ID NO:28 and SEQ ID NO:35).

[0062] The heavy chain variable region may be combined with a suitable human constant region. The light chain variable region may be combined with a suitable constant region.

[0063] The antibody of the invention may comprise a heavy chain of SEQ ID NO:62 and a light chain variable region of SEQ ID NO:64.

[0064] The disc-like SAP molecule has two faces. The single alpha helix present on each of the 5 protomers is located on the A face. The calcium dependent ligand binding pocket of each protomer is located on the B face and this face is therefore occluded when SAP is bound to amyloid fibrils. For an antibody of the present invention to have therapeutic utility, the epitope recognised by the antibody described herein is desirably accessible in SAP when SAP is bound to amyloid deposits and is therefore located on the A face or the edges of the SAP molecule. The antibody can then recognise and bind to amyloid bound SAP, leading to complement activation that triggers the body's efficient macrophage dependent clearance mechanism. Accordingly, in an embodiment of the invention the antibody binds human SAP which is bound to amyloid fibrils *in vivo*. In another embodiment of the invention, the antibody binds to the A face of human SAP.

[0065] The antibody may comprise one or more modifications selected from a mutated constant domain such that the antibody has altered effector functions/ADCC and/or complement activation. Examples of suitable modifications are described in Shields et al. J. Biol. Chem (2001) 276: 6591-6604, Lazar et al. PNAS (2006) 103: 4005-4010 and US6737056, WO2004063351 and WO2004029207.

[0066] The antibody may comprise a constant domain with an altered glycosylation profile such that the antibody has altered effector functions/ADCC and/or complement activation. Examples of suitable methodologies to produce an antibody with an altered glycosylation profile are described in WO2003/011878, WO2006/014679 and EP1229125.

[0067] The present invention also provides a nucleic acid molecule which encodes an antibody as described herein.

[0068] The nucleic acid molecule which encodes the heavy chain variable region may comprise SEQ ID NO:54. The nucleic acid molecule which encodes the light chain variable region may comprise SEQ ID NO:59.

[0069] The nucleic acid molecule may also contain one or more nucleotide substitutions which do not alter the amino acid sequence of the encoded heavy and/or light chain.

[0070] The present invention also provides an expression vector comprising a nucleic acid molecule as described herein. Also provided is a recombinant host cell, comprising an expression vector as described herein.

[0071] The antibody described herein may be produced in a suitable host cell. A method for the production of the antibody as described herein may comprise the step of culturing a host cell as described herein and recovering the antibody. A recombinant transformed, transfected, or transduced host cell may comprise at least one expression cassette, whereby said expression cassette comprises a polynucleotide encoding a heavy chain of the antigen binding protein described herein and further comprises a polynucleotide encoding a light chain of the antibody described herein. Alternatively, a recombinant transformed, transfected or transduced host cell may comprise at least one expression cassette, whereby a first expression cassette comprises a polynucleotide encoding a heavy chain of the antibody described herein and further comprise a second cassette comprising a polynucleotide encoding a light chain of the antibody described herein. A stably transformed host cell may comprise a vector comprising one or more expression cassettes encoding a heavy chain and/or a light chain of the antibody described herein. For example such host cells may comprise a first vector encoding the light chain and a second vector encoding the heavy chain.

[0072] The host cell may be eukaryotic, for example mammalian. Examples of such cell lines include CHO or NS0. The host cell may be cultured in a culture media, for example serum-free culture media. The antibody may be secreted by the host cell into the culture media. The antibody can be purified to at least 95% or greater (e.g. 98% or greater) with respect to said culture media containing the antibody.

[0073] A pharmaceutical composition comprising the antibody and a pharmaceutically acceptable carrier may be provided. A kit-of-parts comprising the pharmaceutical composition together with instructions for use may be provided. For convenience, the kit may comprise the reagents in predetermined amounts with instructions for use.

Antibody Structures

Intact Antibodies

[0074] The light chains of antibodies from most vertebrate species can be assigned to one of two types called Kappa and

Lambda based on the amino acid sequence of the constant region. Depending on the amino acid sequence of the constant region of their heavy chains, human antibodies can be assigned to five different classes, IgA, IgD, IgE, IgG and IgM. IgG and IgA can be further subdivided into subclasses, IgG1, IgG2, IgG3 and IgG4; and IgA1 and IgA2. Species variants exist with mouse and rat having at least IgG2a, IgG2b.

[0075] The more conserved portions of the variable region are called Framework regions (FR). The variable domains of intact heavy and light chains each comprise four FR connected by three CDRs. The CDRs in each chain are held together in close proximity by the FR regions and with the CDRs from the other chain contribute to the formation of the antigen binding site of antibodies.

[0076] The constant regions are not directly involved in the binding of the antibody to the antigen but exhibit various effector functions such as participation in antibody dependent cell-mediated cytotoxicity (ADCC), phagocytosis via binding to Fcγ receptor, half-life/clearance rate via neonatal Fc receptor (FcRn) and complement activation via the C1q component, leading to the chemotactic, opsonic and, potentially in the case of a viable cellular antigen target, cytolytic actions of complement. Human antibodies of the IgG1 class are the most potent in activating the complement system and are therefore the desirable isotype for the therapeutic application of the antibodies of the present invention.

[0077] The human IgG2 constant region has been reported to essentially lack the ability to activate complement by the classical pathway or to mediate antibody-dependent cellular cytotoxicity. The IgG4 constant region has been reported to lack the ability to activate complement by the classical pathway and mediates antibody-dependent cellular cytotoxicity only weakly. Antibodies essentially lacking these effector functions may be termed 'non-lytic' antibodies.

Chimeric and Humanised Antibodies

[0078] Chimeric antibodies are typically produced using recombinant DNA methods. DNA encoding the antibodies (e.g. cDNA) is isolated and sequenced using conventional procedures (e.g. by using oligonucleotide probes that are capable of binding specifically to genes encoding the H and L chains of the antibody. Hybridoma cells serve as a typical source of such DNA. Once isolated, the DNA is placed into expression vectors which are then transfected into host cells such as E. coli, COS cells, CHO cells or myeloma cells that do not otherwise produce immunoglobulin protein to obtain synthesis of the antibody. The DNA may be modified by substituting the coding sequence for human L and H chains for the corresponding non-human (e.g. murine) H and L constant regions, see for example Morrison (1984) PNAS 81: 6851.

[0079] A large decrease in immunogenicity can be achieved by grafting only the CDRs of non-human (e.g. murine) antibodies ("donor" antibodies) onto human framework ("acceptor framework") and constant regions to generate humanised antibodies (see Jones et al. (1986) Nature 321: 522-525; and Verhoeven et al. (1988) Science 239: 1534-1536). However, CDR grafting per se may not result in the complete retention of antigen-binding properties and it is frequently found that some framework residues (sometimes referred to as "back mutations") of the donor antibody need to be preserved in the humanised molecule if significant antigen-binding affinity is to be recovered (see Queen et al. (1989) PNAS 86: 10,029-10,033; Co et al. (1991) Nature 351: 501-502). In this case, human variable regions showing the greatest sequence homology to the non-human donor antibody are chosen from a database in order to provide the human framework (FR). The selection of human FRs can be made either from human consensus or individual human antibodies. Where necessary, key residues from the donor antibody can be substituted into the human acceptor framework to preserve CDR conformations. Computer modelling of the antibody maybe used to help identify such structurally important residues, see WO 99/48523.

[0080] Alternatively, humanisation maybe achieved by a process of "veneering". A statistical analysis of unique human and murine immunoglobulin heavy and light chain variable regions revealed that the precise patterns of exposed residues are different in human and murine antibodies, and most individual surface positions have a strong preference for a small number of different residues (see Padlan et al. (1991) Mol. Immunol. 28: 489-498; and Pedersen et al. (1994) J. Mol. Biol. 235: 959-973). Therefore it is possible to reduce the immunogenicity of a non-human Fv by replacing exposed residues in its framework regions that differ from those usually found in human antibodies. Because protein antigenicity may be correlated with surface accessibility, replacement of the surface residues may be sufficient to render the mouse variable region "invisible" to the human immune system (see also Mark et al. (1994) in Handbook of Experimental Pharmacology Vol. 113: The pharmacology of Monoclonal Antibodies, Springer-Verlag, 105-134). This procedure of humanisation is referred to as "veneering" because only the surface of the antibody is altered, the supporting residues remain undisturbed. Further alternative approaches include that set out in WO04/006955 and the procedure of Humaneering™ (Kalobios) which makes use of bacterial expression systems and produces antibodies that are close to human germline in sequence (Alfenito-M Advancing Protein Therapeutics January 2007, San Diego,

California).

Production Methods

[0081] Antigen binding proteins may be produced in transgenic organisms such as goats (see Pollock et al. (1999) J. Immunol. Methods 231: 147-157), chickens (see Morrow (2000) Genet. Eng. News 20: 1-55, mice (see Pollock et al.) or plants (see Doran (2000) Curr. Opin. Biotechnol. 11: 199-204; Ma (1998) Nat. Med. 4: 601-606; Baez et al. (2000) BioPharm 13: 50-54; Stoger et al. (2000) Plant Mol. Biol. 42: 583-590).

[0082] Antigen binding proteins may also be produced by chemical synthesis. However, antigen binding proteins are typically produced using recombinant cell culturing technology well known to those skilled in the art. A polynucleotide encoding the antigen binding protein is isolated and inserted into a replicable vector such as a plasmid for further cloning (amplification) or expression. One expression system is a glutamate synthetase system (such as sold by Lonza Biologics), particularly where the host cell is CHO or NS0. Polynucleotide encoding the antigen binding protein is readily isolated and sequenced using conventional procedures (e.g. oligonucleotide probes). Vectors that may be used include plasmid, virus, phage, transposons, minichromosomes of which plasmids are typically used. Generally such vectors further include a signal sequence, origin of replication, one or more marker genes, an enhancer element, a promoter and transcription termination sequences operably linked to the antigen binding protein polynucleotide so as to facilitate expression. Polynucleotide encoding the light and heavy chains may be inserted into separate vectors and introduced (for example by transformation, transfection, electroporation or transduction) into the same host cell concurrently or sequentially or, if desired, both the heavy chain and light chain can be inserted into the same vector prior to said introduction.

[0083] Codon optimisation may be used with the intent that the total level of protein produced by the host cell is greater when transfected with the codon-optimised gene in comparison with the level when transfected with the sequence. Several methods have been published (Nakamura et al. (1996) Nucleic Acids Research 24: 214-215; WO98/34640; WO97/11086). Due to the redundancy of the genetic code, alternative polynucleotides to those disclosed herein (particularly those codon optimised for expression in a given host cell) may also encode the antigen binding proteins described herein. The codon usage of the antigen binding protein of this invention therefore can be modified to accommodate codon bias of the host cell such to augment transcript and/or product yield (e.g. Hoekema et al Mol Cell Biol 1987 7(8): 2914-24). The choice of codons may be based upon suitable compatibility with the host cell used for expression.

Signal sequences

[0084] Antigen binding proteins may be produced as a fusion protein with a heterologous signal sequence having a specific cleavage site at the N-terminus of the mature protein. The signal sequence should be recognised and processed by the host cell. For prokaryotic host cells, the signal sequence may be for example an alkaline phosphatase, penicillinase, or heat stable enterotoxin II leaders. For yeast secretion the signal sequences may be for example a yeast invertase leader, α factor leader or acid phosphatase leaders see e.g. WO90/13646. In mammalian cell systems, viral secretory leaders such as herpes simplex gD signal and a native immunoglobulin signal sequence may be suitable. Typically the signal sequence is ligated in reading frame to DNA encoding the antigen binding protein. A murine signal sequence such as that shown in SEQ ID NO: 79 may be used.

Origin of replication

[0085] Origin of replications are well known in the art with pBR322 suitable for most gram-negative bacteria, 2 μ plasmid for most yeast and various viral origins such as SV40, polyoma, adenovirus, VSV or BPV for most mammalian cells. Generally the origin of replication component is not needed for mammalian expression vectors but the SV40 may be used since it contains the early promoter.

Selection marker

[0086] Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins e.g. ampicillin, neomycin, methotrexate or tetracycline or (b) complement auxotrophic deficiencies or supply nutrients not available in the complex media or

(c) combinations of both. The selection scheme may involve arresting growth of the host cell. Cells, which have been successfully transformed with the genes encoding the antigen binding protein, survive due to e.g. drug resistance conferred by the co-delivered selection marker. One example is the DHFR selection marker wherein transformants are cultured in the presence of methotrexate. Cells can be cultured in the presence of increasing amounts of methotrexate to amplify the copy number of the exogenous gene of interest. CHO cells are a particularly useful cell line for the DHFR selection. A further example is the glutamate synthetase expression system (Lonza Biologics). An example of a selection gene for use in yeast is the *trp1* gene, see Stinchcomb et al. (1979) Nature 282: 38.

Promoters

[0087] Suitable promoters for expressing antigen binding proteins are operably linked to DNA/polynucleotide encoding the antigen binding protein. Promoters for prokaryotic hosts include *phoA* promoter, beta-lactamase and lactose promoter systems, alkaline phosphatase, tryptophan and hybrid promoters such as *Tac*. Promoters suitable for expression in yeast cells include 3-phosphoglycerate kinase or other glycolytic enzymes e.g. enolase, glyceraldehyde 3 phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose 6 phosphate isomerase, 3-phosphoglycerate mutase and glucokinase. Inducible yeast promoters include alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, metallothionein and enzymes responsible for nitrogen metabolism or maltose/galactose utilization.

[0088] Promoters for expression in mammalian cell systems include viral promoters such as polyoma, fowlpox and adenoviruses (e.g. adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (in particular the immediate early gene promoter), retrovirus, hepatitis B virus, actin, rous sarcoma virus (RSV) promoter and the early or late Simian virus 40. Of course the choice of promoter is based upon suitable compatibility with the host cell used for expression. A first plasmid may comprise a RSV and/or SV40 and/or CMV promoter, DNA encoding light chain variable region (VL), κ C region together with neomycin and ampicillin resistance selection markers and a second plasmid comprising a RSV or SV40 promoter, DNA encoding the heavy chain variable region (VH), DNA encoding the γ 1 constant region, DHFR and ampicillin resistance markers.

Enhancer element

[0089] Where appropriate, e.g. for expression in higher eukaryotes, an enhancer element operably linked to the promoter element in a vector may be used. Mammalian enhancer sequences include enhancer elements from globin, elastase, albumin, fetoprotein and insulin. Alternatively, one may use an enhancer element from a eukaryotic cell virus such as SV40 enhancer (at bp100-270), cytomegalovirus early promoter enhancer, polyoma enhancer, baculoviral enhancer or murine IgG2a locus (see WO04/009823). The enhancer may be located on the vector at a site upstream to the promoter. Alternatively, the enhancer may be located elsewhere, for example within the untranslated region or downstream of the polyadenylation signal. The choice and positioning of enhancer may be based upon suitable compatibility with the host cell used for expression.

Polyadenylation/termination

[0090] In eukaryotic systems, polyadenylation signals are operably linked to DNA/polynucleotide encoding the antigen binding protein. Such signals are typically placed 3' of the open reading frame. In mammalian systems, non-limiting examples include signals derived from growth hormones, elongation factor-1 alpha and viral (e.g. SV40) genes or retroviral long terminal repeats. In yeast systems non-limiting examples of polyadenylation/termination signals include those derived from the phosphoglycerate kinase (PGK) and the alcohol dehydrogenase 1 (ADH) genes. In prokaryotic systems, polyadenylation signals are typically not required and it is instead usual to employ shorter and more defined terminator sequences. The choice of polyadenylation/termination sequences may be based upon suitable compatibility with the host cell used for expression.

Other methods/elements for enhanced yields

[0091] In addition to the above, other features that can be employed to enhance yields include chromatin remodelling elements, introns and host-cell specific codon modification.

Host cells

[0092] Suitable host cells for cloning or expressing vectors encoding antigen binding proteins are prokaryotic, yeast or higher eukaryotic cells. Suitable prokaryotic cells include eubacteria e.g. enterobacteriaceae such as *Escherichia* e.g. *E. coli* (for example ATCC 31,446; 31,537; 27,325), *Enterobacter*, *Erwinia*, *Klebsiella* *Proteus*, *Salmonella* e.g. *Salmonella typhimurium*, *Serratia* e.g. *Serratia marcescans* and *Shigella* as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (see DD 266 710), *Pseudomonas* such as *P. aeruginosa* and *Streptomyces*. Of the yeast host cells, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* (e.g. ATCC 16,045; 12,424; 24178; 56,500), *Yarrowia* (EP402, 226), *Pichia pastoris* (EP 183 070, see also Peng et al. (2004) *J. Biotechnol.* 108: 185-192), *Candida*, *Trichoderma reesia* (EP 244 234), *Penicillium*, *Tolypocladium* and *Aspergillus* hosts such as *A. nidulans* and *A. niger* are also contemplated.

[0093] Higher eukaryotic host cells include mammalian cells such as COS-1 (ATCC No.CRL 1650) COS-7 (ATCC CRL 1651), human embryonic kidney line 293, baby hamster kidney cells (BHK) (ATCC CRL.1632), BHK570 (ATCC NO: CRL 10314), 293 (ATCC NO.CRL 1573), Chinese hamster ovary cells CHO (e.g. CHO-K1, ATCC NO: CCL 61, DHFR-CHO cell line such as DG44 (see Urlaub et al. (1986) *Somatic Cell Mol. Genet.* 12: 555-556), particularly those CHO cell lines adapted for suspension culture, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells (ATCC CRL-1587), HELA cells, canine kidney cells (ATCC CCL 34), human lung cells (ATCC CCL 75), Hep G2 and myeloma or lymphoma cells e.g. NS0 (see US 5,807,715), Sp2/0, Y0.

[0094] Such host cells may also be further engineered or adapted to modify quality, function and/or yield of the antigen binding protein. Non-limiting examples include expression of specific modifying (e.g. glycosylation) enzymes and protein folding chaperones.

Cell Culturing Methods

[0095] Host cells transformed with vectors encoding antigen binding proteins may be cultured by any method known to those skilled in the art. Host cells may be cultured in spinner flasks, roller bottles or hollow fibre systems but for large scale production that stirred tank reactors are used particularly for suspension cultures. The stirred tankers may be adapted for aeration using e.g. spargers, baffles or low shear impellers. For bubble columns and airlift reactors direct aeration with air or oxygen bubbles may be used. Where the host cells are cultured in a serum free culture media, the media is supplemented with a cell protective agent such as pluronic F-68 to help prevent cell damage as a result of the aeration process. Depending on the host cell characteristics, either microcarriers may be used as growth substrates for anchorage dependent cell lines or the cells may be adapted to suspension culture (which is typical). The culturing of host cells, particularly invertebrate host cells may utilise a variety of operational modes such as fed-batch, repeated batch processing (see Drapeau et al. (1994) *Cytotechnology* 15: 103-109), extended batch process or perfusion culture. Although recombinantly transformed mammalian host cells may be cultured in serum-containing media such as fetal calf serum (FCS), such host cells may be cultured in synthetic serum-free media such as disclosed in Keen et al. (1995) *Cytotechnology* 17: 153-163, or commercially available media such as ProCHO-CDM or UltraCHO™ (Cambrex NJ, USA), supplemented where necessary with an energy source such as glucose and synthetic growth factors such as recombinant insulin. The serum-free culturing of host cells may require that those cells are adapted to grow in serum free conditions. One adaptation approach is to culture such host cells in serum containing media and repeatedly exchange 80% of the culture medium for the serum-free media so that the host cells learn to adapt in serum free conditions (see e.g. Scharfenberg et al. (1995) in *Anima! Cell Technology: Developments towards the 21st century* (Beuvery et al. eds, 619-623, Kluwer Academic publishers).

[0096] Antigen binding proteins secreted into the media may be recovered and purified using a variety of techniques to provide a degree of purification suitable for the intended use. For example the use of antigen binding proteins for the treatment of human patients typically mandates at least 95% purity, more typically 98% or 99% or greater purity (compared to the crude culture medium). Cell debris from the culture media is typically removed using centrifugation followed by a clarification step of the supernatant using e.g. microfiltration, ultrafiltration and/or depth filtration. A variety of other techniques such as dialysis and gel electrophoresis and chromatographic techniques such as hydroxyapatite (HA), affinity chromatography (optionally involving an affinity tagging system such as polyhistidine) and/or hydrophobic interaction chromatography (HIC, see US 5, 429,746) are available. The antibodies, following various clarification steps, can be captured using Protein A or G affinity chromatography. Further chromatography steps can follow such as ion exchange and/or HA chromatography, anion or cation exchange, size exclusion chromatography and ammonium sulphate precipitation. Various virus removal steps may also be employed (e.g. nanofiltration using e.g. a DV-20 filter). Following these various steps, a purified (for example a monoclonal) preparation comprising at least 75mg/ml or greater, or 100mg/ml or greater, of the antigen binding protein is provided. Such preparations are

substantially free of aggregated forms of antigen binding proteins.

[0097] Bacterial systems may be used for the expression of antigen binding fragments. Such fragments can be localised intracellularly, within the periplasm or secreted extracellularly. Insoluble proteins can be extracted and refolded to form active proteins according to methods known to those skilled in the art, see Sanchez et al. (1999) J. Biotechnol. 72: 13-20; and Cupit et al. (1999) Lett Appl Microbiol 29: 273-277.

[0098] Deamidation is a chemical reaction in which an amide functional group is removed. In biochemistry, the reaction is important in the degradation of proteins because it damages the amide-containing side chains of the amino acids asparagine and glutamine. Asparagine is converted to a mixture of isoaspartate and aspartate. Deamidation of glutamine residues occurs at a much lower rate. Deamidation reactions are believed to be one of the factors that can limit the useful lifetime of a protein, they are also one of the most common post-translational modifications occurring during the manufacture of therapeutic proteins. For example, a reduction or loss of *in vitro* or *in vivo* biological activity has been reported for recombinant human DNase and recombinant soluble CD4, whereas other recombinant proteins appear to be unaffected.

Pharmaceutical Compositions

[0099] Purified preparations of an antibody as described herein may be incorporated into pharmaceutical compositions for use in the treatment of the human diseases, disorders and conditions described herein. The terms diseases, disorders and conditions are used interchangeably. The pharmaceutical composition can be used in the treatment of any diseases where amyloid deposits are present in the tissues and contribute to structural and functional damage leading to clinical illness. SAP is always present in all amyloid deposits *in vivo* and the pharmaceutical composition comprising a therapeutically effective amount of the antibody described herein can be used in the treatment of diseases responsive to clearance of amyloid deposits from the tissues.

[0100] The pharmaceutical preparation may comprise an antibody in combination with a pharmaceutically acceptable carrier. The antibody may be administered alone, or as part of a pharmaceutical composition.

[0101] Typically such compositions comprise a pharmaceutically acceptable carrier as known and called for by acceptable pharmaceutical practice, see e.g. Remington's Pharmaceutical Sciences, 16th edition (1980) Mack Publishing Co. Examples of such carriers include sterilised carriers such as saline, Ringers solution or dextrose solution, optionally buffered with suitable buffers to a pH within a range of 5 to 8.

[0102] Pharmaceutical compositions may be administered by injection or continuous infusion (e.g. intravenous, intraperitoneal, intradermal, subcutaneous, intramuscular or intraportal). Such compositions are suitably free of visible particulate matter. Pharmaceutical compositions may also be administered orally, specifically those containing CPHPC.

[0103] Pharmaceutical compositions may comprise between 1mg to 10g of antibody, for example between 5 mg and 1 g of antibody. Alternatively, the composition may comprise between 5 mg and 500 mg, for example between 5 mg and 50 mg.

[0104] Methods for the preparation of such pharmaceutical compositions are well known to those skilled in the art. Pharmaceutical compositions may comprise between 1 mg to 10 g of antibody in unit dosage form, optionally together with instructions for use. Pharmaceutical compositions may be lyophilised (freeze dried) for reconstitution prior to administration according to methods well known or apparent to those skilled in the art. Where antibodies have an IgG1 isotype, a chelator of copper, such as citrate (e.g. sodium citrate) or EDTA or histidine, may be added to the pharmaceutical composition to reduce the degree of copper-mediated degradation of antibodies of this isotype, see EP0612251. Pharmaceutical compositions may also comprise a solubiliser such as arginine base, a detergent/anti-aggregation agent such as polysorbate 80, and an inert gas such as nitrogen to replace vial headspace oxygen.

[0105] Effective doses and treatment regimes for administering the antibody are generally determined empirically and may be dependent on factors such as the age, weight and health status of the patient and disease or disorder to be treated. Such factors are within the purview of the attending physician. Guidance in selecting appropriate doses may be found in e.g. Smith et al (1977) Antibodies in human diagnosis and therapy, Raven Press, New York.

[0106] The dosage of antibody administered to a subject is generally between 1 µg/kg to 150 mg/kg, between 0.1 mg/kg and 100 mg/kg, between 0.5 mg/kg and 50 mg/kg, between 1 and 25 mg/kg or between 1 and 10 mg/kg of the subject's body weight. For example, the dose may be 10 mg/kg, 30 mg/kg, or 60 mg/kg. The antigen binding protein may be administered parenterally, for

example subcutaneously, intravenously or intramuscularly.

[0107] The SAP-depleting compound may be administered at a dose of between 0.1 mg/kg and 2 mg/kg, depending on its activity. The SAP-depleting compound may be administered as a fixed dose, independent of a dose per subject weight ratio. The SAP-depleting compound may be administered in one or more separate, simultaneous or sequential parenteral doses of 100 mg or less, of 50 mg or less, 25 mg or less, or 10 mg or less.

[0108] If desired, the effective daily dose of a therapeutic composition may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

[0109] The antibody may be administered in a single large dose or in smaller repeated doses.

[0110] The administration of a dose may be by slow continuous infusion over a period of from 2 to 24 hours, such as from 2 to 12 hours, or from 2 to 6 hours. This may result in reduced toxic side effects.

[0111] The administration of a dose may be repeated one or more times as necessary, for example, three times daily, once every day, once every 2 days, once a week, once a fortnight, once a month, once every 3 months, once every 6 months, or once every 12 months. The antibody may be administered by maintenance therapy, for example once a week for a period of 6 months or more. The antibody may be administered by intermittent therapy, for example for a period of 3 to 6 months and then no dose for 3 to 6 months, followed by administration of antibody again for 3 to 6 months, and so on in a cycle.

[0112] For example, the dose may be administered subcutaneously, once every 14 or 28 days in the form of multiple sub-doses on each day of administration.

[0113] The antibody may be administered to the subject in such a way as to target therapy to a particular site. For example, the antibody may be injected locally into a circumscribed local amyloid mass in the tissues, or infused into the blood supply to an amyloidotic organ.

[0114] The antigen binding protein must be used in combination with one or more other therapeutically active agents, specifically SAP depleting compounds, for the treatment of the diseases described herein. Effective depletion of SAP from the circulation must be achieved before administration of the SAP binding protein in order for the latter to be given both safely and effectively.

[0115] The SAP depleting compound is administered first so that almost all of the circulating SAP is cleared. Since this leaves substantial amounts of SAP associated with the amyloid deposits in the tissues the sequential administration of an anti-SAP antigen binding protein enables the localisation and specific binding to the amyloid deposits to promote their rapid and extensive regression. Suitably, the anti-SAP antigen binding protein may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20 or 25 or more days after starting the treatment(s) with the SAP depleting compound.

[0116] The sequential administration may involve two or more sequential treatments with SAP depleting compound followed by two or more sequential treatments with the anti-SAP antigen binding protein.

[0117] The sequential administration may involve one treatment with SAP depleting compound followed by one sequential treatment with the anti-SAP antigen binding protein, which is then repeated one or more times.

[0118] The sequential/subsequent dose may be an amount that is more than the initial/previous dose or less than the initial/previous dose.

[0119] The administration of an initial dose of SAP-depleting compound protein may be followed by the administration of one or more sequential (e.g. subsequent) doses of SAP depleting compound and/or the anti-SAP antibody, and wherein said one or more sequential doses may be in an amount that is approximately the same or less than the initial dose.

[0120] After initial depletion of circulating SAP, the administration of further doses of SAP depleting compound and the first dose of anti-SAP antibody may be followed by the administration of one or more sequential (e.g. subsequent) doses, and wherein at least one of the subsequent doses is in an amount that is more than the initial dose.

[0121] Accordingly, the administration may use a pre-determined or routine schedule for administration, thereby resulting in a predetermined designated period of time between dose administrations. The schedule may encompass periods of time which are identical or which differ in length, as long as the schedule is predetermined. Any particular combination would be covered by the

schedule as long as it is determined ahead of time that the appropriate schedule involves administration on a certain day.

[0122] The pharmaceutical composition may comprise a kit of parts of the antibody together with other medicaments, optionally with instructions for use. For convenience, the kit may comprise the reagents in predetermined amounts with instructions for use.

[0123] The terms "individual", "subject" and "patient" are used herein interchangeably. The subject may be a primate (e.g. a marmoset or monkey). The subject is typically a human.

[0124] Treatment can be therapeutic, prophylactic or preventative. The subject will be one who is in need thereof. Those in need of treatment may include individuals already suffering from a particular medical disease in addition to those who may develop the disease in the future.

[0125] Thus, the SAP depleting compound followed by the SAP antibody described herein can be used for prophylactic or preventative treatment. In this case, the sequential treatments described herein are administered to an individual in order to prevent or delay the onset of one or more aspects or symptoms of the disease. The subject can be asymptomatic or may have a genetic predisposition to the disease, as amyloid deposits are known to be present in the tissues and to accumulate for periods of time before they cause sufficient damage to produce clinical symptoms. Such sub-clinical amyloid deposition can be detected by histological examination of tissue biopsies or by non-invasive imaging procedures, including radiolabelled SAP scintigraphy, echocardiography and cardiac magnetic resonance imaging. After first depleting circulating SAP, a prophylactically effective amount of the antibody is administered to such an individual. A prophylactically effective amount is an amount which prevents or delays the onset of one or more aspects or symptoms of a disease described herein.

[0126] The antibody described herein may also be used in methods of therapy. The term "therapy" encompasses alleviation, reduction, or prevention of at least one aspect or symptom of a disease. For example, the antibody described herein may be used to ameliorate or reduce one or more aspects or symptoms of a disease described herein.

[0127] The antibody described herein is used in an effective amount for therapeutic, prophylactic or preventative treatment. A therapeutically effective amount of the antibody described herein is an amount effective to ameliorate or reduce one or more aspects or symptoms of the disease. The antibody described herein may also be used to treat, prevent, or cure the disease described herein.

[0128] The antibody described herein can have a generally beneficial effect on the subject's health, for example it can increase the subject's expected longevity.

[0129] The antibody described herein need not affect a complete cure, or eradicate every symptom or manifestation of the disease to constitute a viable therapeutic treatment. As is recognised in the pertinent field, drugs employed as therapeutic agents may reduce the severity of a given disease state, but need not abolish every manifestation of the disease to be regarded as useful therapeutic agents. Similarly, a prophylactically administered treatment need not be completely effective in preventing the onset of a disease in order to constitute a viable prophylactic agent. Simply reducing the impact of a disease (for example, by reducing the number or severity of its symptoms, or by increasing the effectiveness of another treatment, or by producing another beneficial effect), or reducing the likelihood that the disease will occur (for example by delaying the onset of the disease) or worsen in a subject, is sufficient.

[0130] An antibody described herein may be used in treating or preventing a disease associated with amyloid deposition i.e. amyloidosis.

[0131] "Amyloidosis" is any disease characterized by the extracellular accumulation of amyloid in various organs and tissues of the body.

[0132] The term "amyloid" refers to extracellular deposits in the tissues of insoluble protein fibres composed of fibrils with characteristic ultrastructural morphology, a cross- β sheet core structure and the pathognomonic histochemical tinctorial property of binding Congo red dye from alkaline alcoholic solution and then giving red-green dichroism when viewed microscopically in strong cross polarised light. About 25 different unrelated proteins are known to form amyloid fibrils which deposit in human tissues and share all these typical properties. Amyloid deposits in the brain substance, cerebral amyloid, differ somewhat from amyloid deposits elsewhere in the body in that they are always focal and microscopic in size, and are commonly referred to as amyloid plaques.

[0133] Amyloidosis, that is disease directly caused by deposition of amyloid in the tissues, comprises both local amyloidosis, in which the deposits are confined to one anatomical region and/or one tissue or organ system, and systemic amyloidosis in which the deposits can occur in any organ or tissue in the body, including blood vessels and connective tissues. The cause of amyloidosis can be either acquired or hereditary. Acquired amyloidosis arises as a complication of a preceding medical condition, which can itself be either acquired or hereditary. Thus reactive systemic amyloidosis, known as amyloid A protein (AA) type is a complication of chronic active inflammatory diseases such as rheumatoid arthritis, juvenile rheumatoid arthritis, Crohn's disease, chronic infections and chronic sepsis, and of hereditary periodic fever syndromes such as familial Mediterranean fever, Muckle-Wells syndrome and CINCA syndrome. Dialysis related amyloidosis is caused by accumulation of β 2-microglobulin as a result of end stage renal failure. Monoclonal immunoglobulin light chain (AL) amyloidosis is a complication of multiple myeloma or otherwise benign monoclonal gammopathy (monoclonal gammopathy of uncertain significance, MGUS). Acquired amyloidosis of transthyretin type can occur without any preceding illness and is merely a complication of old age. Hereditary amyloidosis is caused by mutations in the genes for various proteins which encode expression of variant proteins having an increased propensity to form amyloid fibrils, and includes disease caused by transthyretin, apolipoprotein AI, gelsolin, lysozyme, cystatin C and amyloid β -protein. Comprehensive descriptions of all the different forms of amyloidosis and the proteins involved are available in textbooks and the scientific literature (Pepys, M.B. (2006) *Annu. Rev. Med.*, 57: 223-241; Pepys and Hawkins (2003) *Amyloidosis. Oxford Textbook of Medicine*, 4th Ed., Vol. 2, Oxford University Press, Oxford, pp. 162-173; Pepys and Hawkins (2001) *Amyloidosis. Samter's Immunologic Diseases*, Sixth Ed., Vol. 1, Lippincott Williams & Williams, Philadelphia, pp. 401-412).

[0134] Local amyloid deposition, confined to one organ or tissue, can be clinically silent or can cause serious tissue damage and disease. For example, cerebral amyloid angiopathy in which the vascular amyloid deposits are composed of A β protein, is usually a sporadic acquired condition arising for reasons which are not understood in the absence of any other pathology, and is a major cause of cerebral haemorrhage and stroke. There are several very important and common diseases, particularly Alzheimer's disease (AD) and type 2 diabetes, in which amyloid deposits are always present but in which the precise mechanisms causing these respective diseases are not yet known. Nevertheless the local deposition of amyloid in the brain and cerebral blood vessels in Alzheimer's disease, and in the pancreatic islets in diabetes is very likely to exacerbate pathology and disease. Accordingly, the present invention includes treatment of both Alzheimer's disease and type 2 diabetes, indeed of any condition associated with the presence of amyloid deposits in the tissues, with antigen binding proteins as disclosed herein.

[0135] Many forms of transmissible spongiform encephalopathy (prion diseases) are associated with amyloid deposits in the brain, and the present invention therefore relates to all these conditions, including variant Creutzfeldt-Jakob disease in humans, Creutzfeldt-Jakob disease itself, kuru and the various other forms of human prion disease, and also bovine spongiform encephalopathy, chronic wasting disease of mule-deer and elk, and transmissible encephalopathy of mink.

Diagnostic methods of use

[0136] The antibody described herein may be used to detect SAP in a biological sample *in vitro* or *in vivo* for diagnostic purposes. For example, the anti-SAP antibody can be used to detect SAP in serum or in associated with amyloid e.g. amyloid plaques. The amyloid may have been first removed (for example a biopsy) from a human or animal body. Conventional immunoassays may be employed, including ELISA, Western blot, immunohistochemistry, or immunoprecipitation.

[0137] The antibody may be provided in a diagnostic kit comprising one or more antibodies, a detectable label, and instructions for use of the kit. For convenience, the kit may comprise the reagents in predetermined amounts with instructions for use.

EXAMPLES

Example 1- Sequencing of Hybridoma Variable domains: SAP-E and SAP-K

[0138] SAP-E and SAP-K are from two groups of anti-SAP monoclonals, each group having been tested separately for their binding to human SAP *in vitro*. SAP-E and SAP-K showed the strongest binding to SAP, within their groups, and were compared with each other in different assays.

[0139] The first group of antibodies comprised antibodies from 7 hybridomas generated in a single conventional immunization with purified human SAP (SEQ ID NO:43 shown below) (details of method for purifying human SAP are given in Hawkins et al. (1991) *Clin. Exp. Immunol.* 84, 308-316) and fusion protocol and are designated SAP-A to SAP-G. Two of these antibodies, SAP-

E and SAP-B, are IgG2a isotype while the others are all IgG1 isotype (see Example 13, Table 11).

[0140] The second group of antibodies comprised 6 different IgG2a monoclonals (SAP-H to SAP-M) derived by standard techniques from immunization with purified human SAP (SEQ ID NO:43 shown below) (Hawkins et al. (1991) Clin. Exp. Immunol. 84, 308-316) and a conventional fusion to produce hybridomas which were cloned by routine methods.

homo sapiens SAP mature amino acid sequence (SEQ ID NO:43)

[0141]

HTDLSGKVFVFPRESVTDHVNLTIPLEKPLQNFTLCFRAYSDLSTRAYSLFSYNTQGRDNELLVYKERVGEYS
LYIGRHKVTSKVIKFPAPVHICVSWESSSGIAEFWINGTPLVKKGLRQGYFVEAQPKIVLGQEQDSYGGK
FDRSQSFVGEIGDLYMWDSVLPENILSAYQGTPLPANILDWQALNIEIRGYVIKPLVWV

[0142] For comparison purposes, the mouse SAP sequence, which has a 69.4% identity with human SAP, is given below.

mus musculus SAP mature protein (SEQ ID NO:44)

[0143]

QTDLKRKVFVFPRESETHVKLIPHLEKPLQNFTLCFRYSDLSRSQSLFSYSVKGRDNELLYKEKVGESLY
IGQSKVTVRGMEEYLSVHLCITWESSSGIVEFWVNGKPWVKSLQREYTVKAPPSIVLGQEQDNYGG
GFQRSQSFVGEFSDLYMWWDYVLTQDILFVYRDSVPVNPILNWQALNIEINGYVIRPRVW

[0144] Total RNA was extracted from hybridoma cell pellets of approximately 10^6 cells using the RNeasy kit from Qiagen (#74106). AccessQuick RT-PCR System (A1702) was used to produce cDNA of the variable heavy and light regions using degenerate primers specific for the murine immunoglobulin gene leader sequences and murine IgG2a/ κ constant regions. The purified RT-PCR fragments were cloned using the TA cloning kit from Invitrogen (K2000-01). A consensus sequence was obtained for each hybridoma by sequence alignment, and alignment with known immunoglobulin variable sequences listed in KABAT (Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987)). The consensus sequences for SAP-E and SAP-K are shown below.

SAP-E sequences

SAP-E CDRH1 (SEQ ID NO:1)

[0145] TYNMH

SAP-E CDRH2 (SEQ ID NO:2)

[0146] YIYPGDGNANYNQQFKG

SAP-E CDRH3 (SEQ ID NO:3)

[0147] GDFDYDGGYYFDS

SAP-E CDRL1 (SEQ ID NO:4)

[0148] RASENIYSYLA

SAP-E CDRL2 (SEQ ID NO:5)

[0149] NAKTLAE

SAP-E CDRL3 (SEQ ID NO:6)

[0150] QHHYGAPLT

SAP-E V_H amino acid sequence (SEQ ID NO:7) with CDRs underlined

[0151]

QASLQQSGTELVRSVASVKMSCKASGFTFATYNMHWIKQTPGQGLEWIGYIYPGDGNANYNQQFKGK
ATLTADTSSNTAYMQISSLTSEDSAVYFCARGDFDYDGGYYFDSWGQGTTLTVSS

[0152] SAP-E V_H DNA sequence (SEQ ID NO:8)

CAGGCTTCTCTACAGCAGTCTGGGACTGAGCTGGTGAGGTCTGGGGCCTCAGTGAAGATGTCCTGC
AAGGCTTCTGCTTCACATTTGCCACTTACAATATGCACTGGATTAAGCAGACACCCGGACAGGGCC
TGAATGGATTGGGTATATTTATCCTGGAGATGGTAATGCTAACTACAATCAGCAGTTCAAGGGCAA
GGCCACATTGACTGCAGACACATCCTCAACACAGCCTACATGCAGATCAGCAGCCTGACATCTGAA
GACTCTGCGGTCTATTTCTGTGCAAGAGGGGACTTTGATTACGACGGAGGGTACTACTTTGACTCCT
GGGGCCAGGGCACCCTCTCACAGTCTCCTCA

SAP-E V_L amino acid sequence (SEQ ID NO:9) with CDRs underlined

[0153]

DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKQGRSPQLLVHNAKTLAEGVPSRVSGSGSGTH
FSLKINGLQPEDFGNYQCQHHYGAPLTFGAGTKLELK

SAP-E V_L DNA sequence (SEQ ID NO:10)

[0154]

GACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGTGGGAGAACTGTCACCATCACATG
TCGAGCAAGTGAGAAATTTACAGTTATTTAGCATGGTATCAGCAGAAACAGGGAAGATCCCCTCAG
CTCCTGGTCCATAATGCAAAAACCTTAGCAGAAGGTGTGCCATCAAGGGTCAGTGGCAGTGGATCA
GGCACACACTTTTCTCTGAAGATCAACGGCCTGCAGCCTGAAGATTTTGGGAATTATTACTGTCAAC
ATCATTATGGTGCTCCGCTCACGTTCCGGTGCTGGGACCAAGCTGGAAGTGAAG

SAP-K sequences

SAP-K CDRH1 (SEQ ID NO:11)

[0155] SYWMH

SAP-K CDRH2 (SEQ ID NO:12)

[0156] MIHPNSVNTNYNEKFKS

SAP-K CDRH3 (SEQ ID NO:13)

[0157] RNDYYWYFDV

SAP-K CDRL1 (SEQ ID NO:14)

[0158] KASQNVNSNVA

SAP-K CDRL2 (SEQ ID NO:15)

[0159] SASYRYS

SAP-K CDRL3 (SEQ ID NO:16)

[0160] QQCNNYPFT

SAP-K V_H amino acid sequence (SEQ ID NO:17) with CDRs underlined

[0161]
QVQLQQPGAELIKPGASVKLSCKASGYFTSYWMHWVKQRPQGLEWIGMIHPNSVNTNYNEKFKSK
ATLTVDKSSSTAYMQLNSLTSEDSAVVYCARRNDYYWYFDVWGTGTTTVSS

SAP-K V_H DNA sequence (SEQ ID NO:18)

[0162]
CAGGTCCAACCTGCAGCAGCCTGGGGCTGAGCTGATAAAGCCTGGGGCTCAGTGAA⁶TTGTCCTGC
AAGGCTTCTGGCTACACTTTCACCAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGC
CTTGAGTGGATTGGAATGATTCATCCTAATAGTGTTAATACTAACTACAATGAGAAGTTCAAGAGTA
AGGCCCACTGACTGTAGACAAATCCTCCAGCACAGCCTACATGCAACTCAACAGCCTGACATCTGA
GGACTCTGCGGTCTATTACTGTGCAAGACGGAATGATTACTACTGGTACTTCGATGTCTGGGGCACA
GGGACCACGGTCACCGTCTCCTCA

SAP-K V_L amino acid sequence (SEQ ID NO:19) with CDRs underlined

[0163]
DIVMTQSQKFMSTSVGDRVSVTC¹KASQNVNSNVAWYQQKPGQSPKALIYS²SASYRYS³GV⁴PDRFTGSGSG
TDFTLTITNVQSEDLAEYFC⁵QQC⁶NNYPFT⁷FGSGTKLEIK

SAP-K V_L DNA sequence (SEQ ID NO:20)**[0164]**

GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCT
 GCAAGGCCAGTCAGAATGTGAATTCTAATGTAGCCTGGTATCAACAGAAACCAGGGCAATCTCCTAA
 AGCACTGATTTACTCGGCTTCTACCGGTACAGTGGAGTCCCTGATCGCTTCACAGGCAGTGGATCT
 GGGACAGATTTACTCTCACCATCACCAATGTGCAGTCTGAAGACTTGGCAGAGTATTCTGTCAGC
 AATGTAACAACATATCCATTACGTTTCGGCTCGGGACAAAGTTGGAATAAAA

Example 2: Construction of chimeric antibodies

[0165] Chimeric antibodies, comprising parent murine variable domains grafted onto human IgG1/k wild-type constant regions were constructed by PCR cloning for SAP-E and SAP-K. Based on the consensus sequence, primers to amplify the murine variable domains were designed, incorporating restriction sites required to facilitate cloning into mammalian expression vectors. Through introduction of the restriction site in FR4 (Framework Region 4 (V-region sequence following CDR3 and preceding first constant domain)) the V_H amino acid sequence in SAP-E was changed from TTLTVSS as shown in SEQ ID NO:7 to TLVTVSS and the V_H amino acid sequence in SAP-K was changed from TTVTVSS as shown in SEQ ID NO:17 to TLVTVSS. In the SAP-K variable light chain an internal EcoRI site was present in CDRL1 and mutagenesis primers were designed to remove this undesired internal EcoRI site by changing one base pair - this did not change the amino acid sequence.

[0166] The full length heavy and light chain protein sequences of the SAP-E chimeric antibody (cSAP-E) are given in SEQ ID NO:21 and SEQ ID NO:22 respectively. The full length heavy and light chain protein sequences of the SAP-K chimeric antibody (cSAP-K) are given in SEQ ID NO:23 and SEQ ID NO:24 respectively.

SAP-E V_H chimera nucleotide sequence (SEQ ID NO:45)**[0167]**

CAGGCTTCTCTACAGCAGTCTGGGACTGAGCTGGTGAGGTCTGGGGCTCAGTGAAGATGTCCTGC
 AAGGCTTCTGGCTTCACATTTGCCACTTACAATATGCACTGGATTAAGCAGACACCCGGACAGGGCC
 TGGAATGGATTGGGTATATTTATCCTGGAGATGGTAATGCTAACTACAATCAGCAGTTCAAGGGCAA
 GGCCACATTGACTGCAGACACATCTCCAACAGCCTACATGCAGATCAGCAGCCTGACATCTGAA
 GACTCTGCGGTCTATTCTGTGCAAGAGGGGACTTTGATTACGACGGAGGGTACTACTTTGACTCCT
 GGGGCCAGGGCACACTAGTGACCGTGTCAGCGCCAGCACCAAGGGCCCGAGCGTGTCCCCCTGG
 CCCCCAGCAGCAAGAGCACCGCGGGCAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCC
 CCGAACCAGGTGACCGTGTCTGGAACAGCGGAGCCCTGACCAGCGGCGTGACACCTCCCCGCCG
 TGCTGCAGAGCAGCGGCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCAGCAGCAGCCTGGGC
 ACCCAGACCTACATCTGTAACGTGAACCAAGCCAGCAACACCAAGGTGGACAAGAAGGTGGAG
 CCAAGAGCTGTGACAAGACCCACACCTGCCCCCCCTGCCCTGCCCCGAGCTGTGGGAGGCCCCA
 GCGTGTCTCTGTCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCGAGGTGACCTG
 TGTGGTGGTGGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGG
 AGGTGCAATGCCAAGACCAAGCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCC
 GTGCTGACCGTGTGCACAGGATTGGCTGAACGGCAAGGAGTACAAGTGAAGGTGTCCAACAAG
 GCCCTGCCTGCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCAGAGAGCCCGAGGTG
 TACACCCTGCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAG
 GGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAATAACA
 GACCACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAG
 AGCAGATGGCAGCAGGGCAACGTGTTGAGCTGCTCCGTGATGCACGAGGCCCTGCACAATCACTAC
 ACCCAGAAGAGCCTGAGCCTGTCCCTGGCAAG

SAP-E VH chimera amino acid sequence (SEQ ID NO:21)**[0168]**

QASLQQSGTELVRSGASVKMSCKASGFTFATYNMHWIKQTPGQGLEWIGYIYPGDGNANYNQQFKGK
 ATLTADTSSNTAYMQISLTSSEDSAVYFCARGDFDYDGGYFDSWQGGLTVTVSSASTKGPSVFLAPSS
 KTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN
 HKPSNTKVDKKVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
 FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
 REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDK
 SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SAP-E VL chimera nucleotide sequence (SEQ ID NO:46)**[0169]**

GACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGTGGGAGAACTGTCACCATCACATG
 TCGAGCAAGTGAGAAATATTTACAGTTATTTAGCATGGTATCAGCAGAAACAGGGAAGATCCCCTCAG
 CTCCTGGTCCATAATGCAAAAACCTTAGCAGAAGGTGTGCCATCAAGGGTCAGTGGCAGTGATCA
 GGCACACACTTTTCTCTGAAGATCAACGGCCTGCAGCCTGAAGATTTTGGGAATTATTACTGTCAAC
 ATCATTATGGTGCTCCGCTCACGTTCCGGTGCTGGGACCAAGCTGGAAGTAAACGTACGGTGGCCG
 CCCCCAGCGTGTTTCATCTTCCCCCAGCGATGAGCAGCTGAAGAGCGGCACCGCCAGCGTGGTGT
 GTCTGCTGAACAACCTCTACCCCCGGGAGGCCAAGGTGCAGTGGGAAGGTGGACAATGCCCTGCAGA
 GCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAGC
 ACCCTGACCCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGTGAGGTGACCCACCAG
 GGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGC

SAP-E VL chimera amino acid sequence (SEQ ID NO:22)**[0170]**

DIQMTQSPASLSASVGETVTITCRASENIYSYLAWYQQKQGRSPQLLVHNAKTLAEGVPSRVSGSGSGTH
 FSLKINGLQPEDFGNYQCQHHYGAPLTFGAGTKLELKRITVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
 REAKVQWKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNR
 GEC

SAP-K VH chimera nucleotide sequence (SEQ ID NO:47)**[0171]**

CAGGTCCAAGTGCAGCAGCCTGGGGCTGAGCTGATAAAGCCTGGGGCTTCAGTGAAGTTGTCCTGC
 AAGGCTTCTGGCTACACTTTCACAGCTACTGGATGCACTGGGTGAAGCAGAGGCCTGGACAAGGC
 CTTGAGTGGATTGGAATGATTTCCTAATAGTGTAAATACTAACTACAATGAGAAGTTCAAGAGTA
 AGGCCCACTGACTGTAGACAAATCCTCCAGCACAGCCTACATGCAACTCAACAGCCTGACATCTGA
 GGACTCTGCGGTCTATTACTGTGCAAGACGGAATGATTACTACTGGTACTTCGATGTCTGGGGCACA
 GGGACACTAGTGACCGTGTCCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCCTCCCTGGCCCCCAGC
 AGCAAGAGCACCAGCGGGCGGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCCGAACCG
 GTGACCGTGTCTGGAACAGCGGAGCCCTGACCAGCGGCGTGACACCTTCCCCGCCGTGCTGCAG

AGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCAGCAGCAGCCTGGGCACCCAGAC
 CTACATCTGTAACGTGAACCAACAAGCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCCCAAGA
 GCTGTGACAAGACCCACACCTGCCCCCTGCCCTGCCCCGAGCTGCTGGGAGGCCCCAGCGTGT
 CCTGTTCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCGAGGTGACCTGTGTGGTG
 GTGGATGTGAGCCACGAGGACCTGAGGTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
 CAATGCCAAGACCAAGCCAGGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGTCCGTGCTGAC
 CGTGTGCAACGAGGATTGGCTGAACGGCAAGGAGTACAAGTGAAGGTGTCCAACAAGGCCCTGCC
 TGCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCAGAGAGCCCCAGGTGTACACCCT
 GCCCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGGGCTTCTA
 CCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCGAGAACAACTACAAGACCACCC
 CCCCTGTGCTGGACAGCGATGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGGACAAGAGCAGAT
 GGCAGCAGGGCAACGTGTTCAGCTGTCCGTGATGCACGAGGCCCTGCACAATCACTACACCCAGA
 AGAGCCTGAGCCTGTCCCCTGGCAAG

SAP-K VH chimera amino acid sequence (SEQ ID NO:23)

[0172]

QVQLQQPGAELIKPGASVKLSCKASGYFTSYWMHWVKRPGQGLEWIGMIHPNSVNTNYNEFKSK
 ATLTVDKSSSTAYMLNSLTSEDSAVYYCARRNDYYWYFDVWGTGLTVSSASTKGPSVFPLAPSSKST
 SGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKP
 SNTKVDKKVEPKSDKTHCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
 PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFCFVMHEALHNHYTQKSLSLSPGK

SAP-K VL chimera nucleotide sequence (SEQ ID NO:48)

[0173]

GACATTGTGATGACCCAGTCTCAAAAATTCATGTCCACATCAGTAGGAGACAGGGTCAGCGTCACCT
 GCAAGGCCAGTCAGAATGTGAACCTAATGTAGCCTGGTATCAACAGAAACCAGGGCAATCTCCTA
 AAGCACTGATTTACTCGGCTTCTACCGGTACAGTGGAGTCCCTGATCGCTTCAAGGCAGTGGATC
 TGGGACAGATTTCACTCTACCATCACCAATGTGCAGTCTGAAGACTTGGCAGAGTATTTCTGTCAG
 CAATGTAACAACTATCCATTACGTTCCGGCTCGGGGACAAAGTTGGAAATAAACGTACGGTGGCC
 GCCCCAGCGTGTTCATCTTCCCCCAGCGATGAGCAGCTGAAGAGCGGCACCGCCAGCGTGGTG
 TGTCTGCTGAACAACTTCTACCCCCGGAGGCCAAGGTGAGTGAAGGTGGACAATGCCCTGCAG
 AGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGCAG
 CACCTGACCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGTGAGGTGACCCACCA
 GGGCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAGTGC

SAP-K VL chimera amino acid sequence (SEQ ID NO:24)

[0174]

DIVMTQSQKFMSTSVGDRVSVTCKASQNVNSNVAWYQQKPGQSPKALIYSASYRYSVGPDRFTGSGSG
 TDFLTITNVQSEDLAEYFCQQCNYPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNIFY
 PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFN
 RGEK

Example 3: Humanisation strategy

[0175] Humanised antibodies were generated by a process of grafting CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3 from the murine antibody onto a suitable human framework sequence.

SAP-E Humanisation Strategy**SAP-E Heavy chain humanisation**

[0176] For the SAP-E mouse variable heavy chain sequence a human germ line acceptor framework was selected (IGHV1-69, SEQ ID NO:25) which had 60% identity (including CDRs) with the mouse SAP-E variable heavy chain sequence (SEQ ID NO:7) together with the JH1 minigene (Kabat: AEYFQHWGQGTLTVSS (SEQ ID NO:26)). The first six residues of the JH1 minigene residues fall within the CDR3 region and were replaced by the incoming CDR from the donor antibody.

[0177] Five humanised variants were generated on the basis of sequence comparison and possible impact on antibody function. Construct H0 was a straight graft of murine CDRs (using the Kabat definition) into the human acceptor framework selected above. Construct H1 has additional back-mutations at residues 27 and 30. Constructs H2 and H3 were based on H1 with additional back-mutations at residues 2 (H2), and 48 and 67 (H3). Construct H4 was based on H3 with additional back-mutations at residues 69, 73 and 91. See Table 3.

[0178] The sequences of the humanised variable heavy domains of H0, H1, H2, H3 and H4 are given below (SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30 and SEQ ID NO:31 respectively).

Table 3: Summary of SAP-E humanised VH variants generated

Construct	Acceptor/template Framework	Back-mutations@ aa# (Kabat)	Total number of back-mutations	Human acceptor framework	Original mouse sequence
H0 (SEQ ID NO:27)	IGHV1-69 (SEQ ID NO:25)	—	NONE	—	—
H1 (SEQ ID NO:28)	H0	27	2	G	F
		30		S	A
H2 (SEQ ID NO:29)	H1	2	3	V	A
H3 (SEQ ID NO:30)	H1	48	4	M	I
		67		V	A
H4 (SEQ ID NO:31)	H3	69	7	I	L
		73		K	T
		91		Y	F

SAP-E Light chain humanisation

[0179] For the SAP-E mouse variable light chain sequence a human germ line acceptor framework was selected (IGKV1-39, SEQ ID NO:32) which had 68% identity (including CDRs) with the mouse SAP-E variable light chain sequence (SEQ ID NO:9) together with the J-region kappa 2 minigene (Kabat: YTFGQGKLEIK, SEQ ID NO:33)) based on sequence similarity. The first two residues of the JK-2 minigene residues fall within the CDR3 region and were replaced by the incoming CDR from the donor antibody.

[0180] Three humanised variants were generated on the basis of sequence comparison and possible impact on antibody function. Construct L0 was a straight graft of murine CDRs (using the Kabat definition) into the human acceptor framework selected above. Construct L1 has a back-mutation at residue 49 and construct L2 has back mutations at positions 48 and 49. See Table 4.

[0181] The sequences of the humanised variable light domains of L0, L1 and L2 are given below (SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36 respectively).

Table 4: Summary of SAP-E humanised VL variants generated

Construct	Acceptor/template Framework	Back-mutations@ aa# (Kabat)	Total number of back-mutations	Human acceptor framework	Original mouse sequence
L0 (SEQ ID NO:34)	IGKV1-39 (SEQ ID NO:32)	—	NONE	—	—
L1 (SEQ ID NO:35)	L0	49	1	Y	H
L2 (SEQ ID NO:36)	L1	48	2	I	V
		49		Y	H

SAP-K Humanisation Strategy

SAP-K Heavy chain humanisation

[0182] For the SAP-K mouse variable heavy chain sequence a human germ line acceptor framework was selected (IGHV1-69, SEQ ID NO:25) which had 65% identity (including CDRs) with the mouse SAP-K variable heavy chain sequence (SEQ ID NO:17) together with the JH1 minigene (Kabat: AEYFQHWGQGTLTVSS (SEQ ID NO:26)). The first six residues of the JH1 minigene residues fall within the CDR3 region and were replaced by the incoming CDR from the donor antibody.

[0183] Four humanised variants were generated on the basis of sequence comparison and possible impact on antibody function. Construct H0 was a straight graft of murine CDRs (using the Kabat definition) into the human acceptor framework selected above. Construct H1 has additional back-mutations at residues 27 and 30. Construct H2 was based on H1 with additional back-mutations at residues 48 and 67. Construct H3 was based on H2 with additional back-mutations at residues 69 and 71. See Table 5.

[0184] The sequences of the humanised variable heavy domains of H0, H1, H2 and H3 are given below (SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39 and SEQ ID NO:40 respectively).

Table 5: Summary of SAP-K humanised VH variants generated

Construct	Acceptor/template Framework	Back-mutations@ aa# (Kabat)	Total number of back-mutations	Human acceptor framework	Original mouse sequence
H0 (SEQ ID NO:37)	IGHV1-69 (SEQ ID NO: 25)	—	NONE	—	—
H1 (SEQ ID NO:38)	H0	27	2	G	Y
		30		S	T
H2 (SEQ ID NO:39)	H1	48	4	M	I
		67		V	A
H3 (SEQ ID NO:40)	H2	69	6	I	L
		71		A	V

SAP-K Light chain humanisation

[0185] For the SAP-K mouse variable light chain sequence a human germ line acceptor framework was selected (IGKV1-39, SEQ ID NO:32) which had 63% identity (including CDRs) with the mouse SAP-K variable light chain sequence (SEQ ID NO:19) together with the J-region kappa 2 minigene (Kabat: YTFGQGTKLEIK, SEQ ID NO:33) based on sequence similarity. The first two residues of the JK-2 minigene residues fall within the CDR3 region and were replaced by the incoming CDR from the donor antibody.

[0186] Two humanised variants were generated on the basis of sequence comparison and possible impact on antibody function. Construct L0 was a straight graft of murine CDRs (using the Kabat definition) into the human acceptor framework selected above. Construct L1 has a back-mutation at residue 46.

[0187] The sequences of the humanised variable light domains of L0 and L1 are given below (SEQ ID NO:41 and SEQ ID NO:42 respectively).

Table 6: Summary of SAP-K humanised VL variants generated

Construct	Acceptor/template Framework	Back-mutations@ aa# (Kabat)	Total number of back-mutations	Human acceptor framework	Original mouse sequence
L0 (SEQ ID NO:41)	IGKV1-39 (SEQ ID NO:32)	—	NONE	—	—
L1 (SEQ ID NO:42)	L0	46	1	L	A

Construction of humanised antibody vectors

[0188] The humanised variable region DNA sequences were sequence optimised. DNA fragments encoding the humanised variable heavy and variable light regions were constructed de novo using a PCR-based strategy and overlapping oligonucleotides. The PCR product was cloned into mammalian expression vectors containing the human gamma 1 constant region and the human kappa constant region respectively. This is the wild-type Fc region.

IGHV1-69 human variable heavy chain germline acceptor nucleotide sequence (SEQ ID NO:49)

[0189]

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCTCGGTGAAGGTCTCCTG
CAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACAGGCCCTGGACAAGG
GCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAACTACGCACAGAAGTTCCAGGG
CAGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATC
TGAGGACACGGCCGTGTATTACTGTGCGAGA

IGHV1-69 human variable heavy chain germline acceptor amino acid sequence (SEQ ID NO:25)

[0190]

QVQLVQSGAEVKKPGSSVKVSKASGGTFSYIAISWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRV
TITADKSTSTAYMELSSLRSEDAVYYCAR

IGKV1-39 human variable heavy chain germline acceptor nucleotide sequence (SEQ ID NO:50)

[0191]

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
CCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAA
GCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCT
GGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTGCAACTTACTACTGTCAACA
GAGTTACAGTACCCCT

IGKV1-39 human variable heavy chain germline acceptor amino acid sequence (SEQ ID NO:32)

[0192]

DIQMTQSPSSLSASVGDRTITCRASQSISSYLNWYQQKPKAPKLLIYAASSLQSGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQSYSTP

JH1 minigene (SEQ ID NO:26)

[0193] AEYFQHWGQGTLVTVSS

Jk2 minigene (SEQ ID NO:33)

[0194] YTFGQGTKLEIK

SAP-E humanised heavy chain V region variant H0 nucleotide sequence non-codon optimised (SEQ ID NO:51)

[0195]

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCTCGGTGAAGGTCTCCTG
CAAGGCTTCTGGAGGCACCTTCAGCACTTACAATATGCACTGGGTGCGACAGGCCCTGGACAAGG
GCTTGAGTGGATGGGATATTTATCCTGGAGATGGTAATGCTAACTACAATCAGCAGTTCAAGGGC
AGAGTCACGATTACCGCGGACAAATCCACGAGCAGCCTACATGGAGCTGAGCAGCCTGAGATCT
GAGGACACGGCCGTGTATTACTGTGCGAGAGGGGACTTTGATTACGACGGAGGGTACTACTTTGAC
TCCTGGGGCCAGGGCACCTGGTCACCGTCTCCTCA

SAP-E humanised light chain V region variant L0 nucleotide sequence non-codon optimised (SEQ ID NO:52)

[0196]

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
CCGAGCAAGTGAGAATATTTACAGTTATTTAGCATGGTATCAGCAGAAACCAGGGAAAGCCCCTAA
GCTCCTGATCTATAATGCAAAAACCTTAGCAGAAGGGGTCCCATCAAGGTTCAAGTGGCAGTGGATCT
GGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTGCAACTTACTACTGTCAACA
TCATTATGGTGTCTCCGCTACGTTTGGCCAGGGGACCAAGCTGGAGATCAA

SAP-E humanised heavy chain V region variant H0 nucleotide sequence (codon optimised) (SEQ ID NO:53)

[0197]

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAACCCGGCAGCAGCGTGAAGGTGAGCT
GCAAGGCTAGCGGGGACCTTCTCCACCTACAACATGCACTGGGTGAGGCAGGCACCCGGCCAGG
GCCTGGAGTGGATGGGCTATATCTACCCGCGGACGGCAACGCCAACTACAACCAGCAGTTCAAGG
GCAGGGTGACCATCACCGCCGACAAGAGCACCAGCACCCTACATGGAAGTGAAGCAGCCTGAGG
AGCGAGGATACCGCGGTGTACTACTGCGCCAGGGGCGACTTCGACTACGACGGCGGCTACTACTTC
GACAGCTGGGGACAGGGCACACTAGTGACCGTGTCCAGC

SAP-E humanised heavy chain V region variant H0 amino acid sequence (SEQ ID NO:27)

[0198]

QVQLVQSGAEVKKPGSSVKVSKASGFTSTYNNMHWVRQAPGQGLEWMGYIYPGDGNANYNQYFK
GRVTITADKSTSTAYMELSSLRSEDAVYYCARGDFDYDGGYYFDSWGQGLTVTVSS

SAP-E humanised heavy chain V region variant H1 nucleotide sequence (codon optimised) (SEQ ID NO:54)

[0199]

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAACCCGGCAGCAGCGTGAAGGTGAGCT
GCAAGGCTAGCGGGTTCACCTTCGCCACCTACAACATGCACTGGGTGAGGCAGGCACCCGGCCAGG
GCCTGGAGTGGATGGGCTATATCTACCCGCGGACGGCAACGCCAACTACAACCAGCAGTTCAAGG
GCAGGGTGACCATCACCGCCGACAAGAGCACCAGCACCCTACATGGAAGTGAAGCAGCCTGAGG
AGCGAGGATACCGCGGTGTACTACTGCGCCAGGGGCGACTTCGACTACGACGGCGGCTACTACTTC
GACAGCTGGGGACAGGGCACACTAGTGACCGTGTCCAGC

SAP-E humanised heavy chain V region variant H1 amino acid sequence (SEQ ID NO:28)

[0200]

QVQLVQSGAEVKKPGSSVKVSKASGFTFATYNNMHWVRQAPGQGLEWMGYIYPGDGNANYNQYFK
GRVTITADKSTSTAYMELSSLRSEDAVYYCARGDFDYDGGYYFDSWGQGLTVTVSS

SAP-E humanised heavy chain V region variant H2 nucleotide sequence (codon optimised) (SEQ ID NO:55)

[0201]

CAGGCGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAACCCGGCAGCAGCGTGAAGGTGAGCT
GCAAGGCTAGCGGGTTCACCTTCGCCACCTACAACATGCACTGGGTGAGGCAGGCACCCGGCCAGG
GCCTGGAGTGGATGGGCTATATCTACCCGCGGACGGCAACGCCAACTACAACCAGCAGTTCAAGG
GCAGGGTGACCATCACCGCCGACAAGAGCACCAGCACCCTACATGGAAGTGAAGCAGCCTGAGG
AGCGAGGATACCGCGGTGTACTACTGCGCCAGGGGCGACTTCGACTACGACGGCGGCTACTACTTC
GACAGCTGGGGACAGGGCACACTAGTGACCGTGTCCAGC

SAP-E humanised heavy chain V region variant H2 amino acid sequence SEQ ID NO:29

[0202]

QAQLVQSGAEVKKPGSSVKVSKASGFTFATYNNMHWVRQAPGQGLEWMGYIYPGDGNANYNQYFK
GRVTITADKSTSTAYMELSSLRSEDAVYYCARGDFDYDGGYYFDSWGQGLTVTVSS

SAP-E humanised heavy chain V region variant H3 nucleotide sequence (codon optimised) (SEQ ID NO:56)

[0203]

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAACCCGGCAGCAGCGTGAAGGTGAGCT
GCAAGGCTAGCGGGTTCACCTTCGCCACCTACAACATGCACTGGGTGAGGCAGGCACCCGGCCAGG
GCCTGGAGTGGATCGGCTATATCTACCCCGGCGACGGCAACGCCAACTACAACCAGCAGTTCAAGG
GCAGGGCCACCATCACCGCCGACAAGAGCACCAGCACCAGCCTACATGGAAGTGAAGCAGCCTGAGGA
GCGAGGATACCGCCGTGTACTACTGCGCCAGGGGCGACTTCGACTACGACGGCGGCTACTACTTCG
ACAGCTGGGGACAGGGCACACTAGTGACCGTGTCAGC

SAP-E humanised heavy chain V region variant H3 amino acid sequence (SEQ ID NO:30)

[0204]

QVQLVQSGAEVKKPGSSVKVSCASGFTFATYNMHWVRQAPGQGLEWIGYIYPGDGNANYNQYFKG
RATITADKSTSTAYMELSSLRSEDAVYYCARGDFDYDGGYFDSWVGQGLTVTVSS

SAP-E humanised heavy chain V region variant H4 nucleotide sequence (codon optimised) (SEQ ID NO:57)

[0205]

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAGGTGAAGAAACCCGGCAGCAGCGTGAAGGTGAGCT
GCAAGGCTAGCGGGTTCACCTTCGCCACCTACAACATGCACTGGGTGAGGCAGGCACCCGGCCAGG
GCCTGGAGTGGATCGGCTATATCTACCCCGGCGACGGCAACGCCAACTACAACCAGCAGTTCAAGG
GCAGGGCCACCTGACCGCCGACACCAGCACCAGCACCAGCCTACATGGAAGTGAAGCAGCCTGAGGA
GCGAGGATACCGCCGTGTACTTCTGCGCCAGGGGCGACTTCGACTACGACGGCGGCTACTACTTCG
ACAGCTGGGGACAGGGCACACTAGTGACCGTGTCAGC

SAP-E humanised heavy chain V region variant H4 amino acid sequence (SEQ ID NO:31)

[0206]

QVQLVQSGAEVKKPGSSVKVSCASGFTFATYNMHWVRQAPGQGLEWIGYIYPGDGNANYNQYFKG
RATLTADTSTSTAYMELSSLRSEDAVYFCARGDFDYDGGYFDSWVGQGLTVTVSS

SAP-E humanised light chain V region variant L0 nucleotide sequence (codon optimised) (SEQ ID NO:58)

[0207]

GACATCCAGATGACCCAGAGCCCCAGCTCACTGAGCGCCAGCGTGGGCGACAGGGTGACCATTACC
TGCAGGGCTCCGAGAACATCTACAGCTACCTGGCTGGTACCAGCAGAAGCCCGGCAAGGCCCCC
AAGCTGCTGATCTACAACGCCAAGACCCTCGCCGAGGGCGTCCCTAGCAGGTTCTCTGGAAGCGGC
AGCGGCACCGACTTCACCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTATTACTGCC
AGCACTACTAGCGCGCCCCCTGACCTTTGGCCAGGGCACCAAACTGGAGATCAAG

SAP-E humanised light chain V region variant L0 amino acid sequence SEQ ID NO:34

[0208]

DIQMTQSPSSLSASVGDRTITCRASENIYSYLAWYQQKPGKAPKLLIYNAKTLAEGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQHHYGAPLTFGQGTKLEIK

SAP-E humanised light chain V region variant L1 nucleotide sequence (codon optimised) (SEQ ID NO:59)

[0209]

GACATCCAGATGACCCAGAGCCCCAGCTCACTGAGCGCCAGCGTGGGCGACAGGGTGACCATTACC
TGCAGGGCCTCCGAGAACATCTACAGCTACCTGGCCTGGTACCAGAGAAGCCCGCAAGGCCCCC
AAGCTGCTGATCCACAACGCCAAGACCCCTGCCGAGGGCGTCCCTAGCAGGTTCTCTGGAAGCGGC
AGCGGCACCGACTTCACCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTATTACTGCC
AGCACCACTACGGCGCCCCCTGACCTTTGGCCAGGGCACCAAACTGGAGATCAAG

SAP-E humanised light chain V regions variant L1 amino acid sequence (SEQ ID NO:35)

[0210]

DIQMTQSPSSLSASVGDRTITCRASENIYSYLAWYQQKPGKAPKLLIYNAKTLAEGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCQHHYGAPLTFGQGTKLEIK

SAP-E humanised light chain V region variant L2 nucleotide sequence (codon optimised) (SEQ ID NO:60)

[0211]

GACATCCAGATGACCCAGAGCCCCAGCTCACTGAGCGCCAGCGTGGGCGACAGGGTGACCATTACC
TGCAGGGCCTCCGAGAACATCTACAGCTACCTGGCCTGGTACCAGAGAAGCCCGCAAGGCCCCC
AAGCTGCTGGTGCACAACGCCAAGACCCCTGCCGAGGGCGTCCCTAGCAGGTTCTCTGGAAGCGGC
AGCGGCACCGACTTCACCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACCTATTACTGCC
AGCACCACTACGGCGCCCCCTGACCTTTGGCCAGGGCACCAAACTGGAGATCAAG

SAP-E humanised light chain V region variant L2 amino acid sequence (SEQ ID NO:36)

[0212]

DIQMTQSPSSLSASVGDRTITCRASENIYSYLAWYQQKPGKAPKLLVHNAKTLAEGVPSRFSGSGSGTD
FTLISSLQPEDFATYYCQHHYGAPLTFGQGTKLEIK

SAP-E humanised heavy chain H1 full mature nucleotide sequence (codon optimised) (SEQ ID NO:61)

[0213]

CAGGTGCAGCTGGTGCAGAGCGGCGCGAGGTGAAGAAACCCGGCAGCAGCGTGAAGGTGAGCT
 GCAAGGCTAGCGGGTTCACCTTCGCCACCTACAACATGCACTGGGTGAGGCAGGCACCCGGCCAGG
 GCCTGGAGTGGATGGGTATATCTACCCGGCGACGGCAACGCCAACTACAACCAGCAGTTCAAGG
 GCAGGGTGACCATCACCGCCGACAAGAGCACCAGCACCCTACATGGAAGTGAAGCAGCCTGAGG
 AGCGAGGATACCGCGTGTACTACTGCGCCAGGGGCGACTTCGACTACGACGGCGGCTACTACTTC
 GACAGCTGGGGACAGGGCACACTAGTGACCGTGTCCAGCGCCAGCACCAGGGCCCCAGCGTGTT
 CCCCCTGGCCCCAGCAGCAAGAGCACCAGCGGCGCACAGCCGCTGGGCTGCCTGGTGAAGG
 ACTACTTCCCCGAACCGGTGACCGTGTCTGGAACAGCGGAGCCCTGACCAGCGGCGTGCACACCTT
 CCCCCTGGTGTGCAGAGCAGCGGCTGTACAGCCTGAGCAGCGTGGTGACCGTCCCCAGCAGCAG
 CCTGGGCACCCAGACCTACATCTGTAACGTGAACCAAGCCAGCAACACCAAGGTGGACAAGAA
 GGTGGAGCCCCAAGAGCTGTGACAAGACCCACACCTGCCCTGCCCCCTGCCCTGCCCTGAGCTGCTGGG
 AGGCCCCAGCGTGTCTGTTCCTCCCCCAAGCCTAAGGACACCTGATGATCAGCAGAACCCCCGAG
 GTGACCTGTGTGGTGGTGGATGTGAGCCACGAGGACCTGAGGTGAAGTTCAACTGGTACGTGGA
 CGGCGTGGAGGTGCACAATGCCAAGACCAAGCCAGGGAGGAGCAGTACAACAGCACCTACCGGG
 TGGTGTCCGTGTGACCGTGTGACCCAGGATTGGCTGAACGGCAAGGAGTACAAGTGAAGGTGT
 CCAACAAGGCCCTGCCTGCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCAGAGAGC
 CCCAGGTGTACACCTGCCCTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCT
 GGTGAAGGGCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCGAGAACA
 ACTACAAGACCACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTCTGTACGCAAGCTGACCGT
 GGACAAGAGCAGATGGCAGCAGGGCAACGTGTTAGCTGCTCCGTGATGCACGAGGCCCTGCACA
 ATCACTACACCCAGAAGAGCCTGAGCCTGTCCCCTGGCAAG

SAP-E humanised heavy chain H1 full mature amino acid sequence (SEQ ID NO:62)

[0214]

QVQLVQSGLAEVKKPGSSVKVSKASGFTFATYNMHWVRQAPGQGLEWMGYIYPGDGNANYNQQFK
 GRVTITADKSTSTAYMELSLRSEDVAVYYCARGDFDYDGGYYFDSWQGLTVVSSASTKGPSVFPLAP
 SSKSTSGGTAAALGLVKDYFPEPVTWSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQYICN
 VNHKPSNTKVDKKVEPKSDKHTCTPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
 VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
 QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTV
 DKSRWQQGNVFSVMHEALHNHYTQKSLSLSPGK

SAP-E humanised light chain L1 full mature nucleotide sequence (codon optimised) (SEQ ID NO:63)

[0215]

GACATCCAGATGACCCAGAGCCCCAGCTCACTGAGCGCCAGCGTGGGCGACAGGGTGACCATTAAC
 TGCAGGGCCTCCGAGAACATCTACAGCTACCTGGCCTGGTACCAGCAGAAGCCCGGCAAGGCCCC
 AAGCTGCTGATCCACAACGCCAAGACCCTGCCGAGGGCGTCCCTAGCAGGTTCTCTGGAAGCGGC
 AGCGGCACCGACTTACCCTGACCATCAGCAGCCTGCAGCCGAGGACTTCGCCACCTATTACTGCC
 AGCACCACTACGGCGCCCCCTGACCTTTGGCCAGGGCACCAACTGGAGATCAAGCGTACGGTGG
 CCGCCCCAGCGTGTTCATCTTCCCCCAGCGATGAGCAGCTGAAGAGCGGCACCGCCAGCGTGG
 TGTGTCTGCTGAACAATTCTACCCCGGGAGGCCAAGGTGCAAGTGAAGGTGGACAATGCCCTGC
 AGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAGC
 AGCACCTGACCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGTGAGGTGACCCAC
 CAGGGCCTGTCCAGCCCCGTGACCAAGAGCTCAACCGGGGCGAGTGC

SAP-E humanised light chain L1 full mature amino acid sequence (SEQ ID NO:64)**[0216]**

DIQMTQSPSSLSASVGDRVTITCRASENIYSYLAWYQQKPGKAPKLLIHNAKTLAEGVPSRFSGSGSGTDF
 TLTISSLPEDFATYYCQHHYGAPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
 KVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

SAP-K humanised heavy chain V region variant H0 nucleotide sequence non-codon optimised (SEQ ID NO:65)**[0217]**

CAGGTGCAGCTGGTGAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCTCGGTGAAGGTCTCCTG
 CAAGGCTTCTGGAGGCACCTTCAGCAGCTACTGGATGCACTGGGTGCGACAGGCCCTGGACAAGG
 GCTTGAGTGGATGGGAATGATTCCTAATAGTGTTAATACTAACTACAATGAGAAGTCAAGAGT
 AGAGTCACGATTACCGCGGACAAATCCACGAGCACAGCCTACATGGAGCTGAGCAGCCTGAGATCT
 GAGGACACGGCCGTATTACTGTGCGAGACGGAATGATTACTACTGGTACTTCGATGTCTGGGGC
 CAGGGCACCTGGTCACCGTCTCCTCA

SAP-K humanised light chain V region variant L0 nucleotide sequence non-codon optimised (SEQ ID NO:66)**[0218]**

GACATCCAGATGACCCAGTCTCCATCCTCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTG
 CAAGGCCAGTCAGAAATGTGAAGTCTAATGTAGCCTGGTATCAGCAGAAACCAGGGAAAGCCCTAA
 GCTCTGATCTATTCGGCTTCTACCGGTACAGTGGGGTCCCATCAAGGTTCACTGGCAGTGGATCT
 GGGACAGATTCTACTCTACCATCAGCAGTCTGCAACCTGAAGATTTGCAACTTACTACTGTGAGCA
 ATGTAACAACTATCCATTACGTTTGCCAGGGGACCAAGCTGGAGATCAA

SAP-K humanised heavy chain V region variant H0 nucleotide sequence (codon optimised) (SEQ ID NO:67)**[0219]**

CAGGTGCAGCTGGTGAGAGCGGCGCCGAAGTGAAGAAGCCCGGCAGCAGCGTGAAGTGAGCT
 GCAAGGCCAGCGGCGGAACCTTCAGCAGCTACTGGATGCACTGGGTGAGGCAGGCACCCGGCCAG
 GGCTTGAGTGGATGGGCATGATCCACCCCAACAGCGTGAACACCACTACAACGAGAAGTTCAAG
 AGCAGAGTGACCATCACCGCCGACAAGAGCACCAGCACCCTATATGGAGCTGAGCTCTCTGAGG
 AGCGAGGATACCGCGGTGTACTACTGCGCCAGGAGGAACGACTACTGCTACTTCGACGTCTGG
 GGCCAGGGCACACTAGTGACCGTGTCCAGC

SAP-K humanised heavy chain V region variant H0 amino acid sequence (SEQ ID NO:37)**[0220]**

QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYWMHWRQAPGQGLEWMGMIHPNSVNTNYNEKFK
 SRVTITADKSTSTAYMELSSLRSEDAVYYCARRNDYYWYFDVWGQGLTVTVSS

SAP-K humanised heavy chain V region variant H1 nucleotide sequence (codon optimised) (SEQ ID NO:68)

[0221]

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAAGTGAAGAAGCCCGGCAGCAGCGTGAAAGTGAGCT
 GCAAGGCCAGCGGTACACCTTCACCAGCTACTGGATGCACTGGGTGAGGCAGGCACCCGGCCAG
 GGCCTGGAGTGGATGGGCATGATCCACCCCAACAGCGTGAACACCAACTACAACGAGAAGTTCAAG
 AGCAGAGTGACCATCACCGCCGACAAGAGCACCAGCACCGCCTATATGGAGCTGAGCTCTCTGAGG
 AGCGAGGATACCGCGTGTACTACTGCGCCAGGAGGAACGACTACTACTGGTACTTCGACGTCTGG
 GGCCAGGGCACACTAGTGACCGTGTCCAGC

SAP-K humanised heavy chain V region variant H1 amino acid sequence (SEQ ID NO:38)

[0222]

QVQLVQSGAEVKKPGSSVKVSKASGYTFTSYWMHWVRQAPGQGLEWMGMIHPNSVNTNYNEKFK
 SRVTITADKSTSTAYMELSLRSEDVAVYVCARRNDYYWYFDVWGQGLTVTVSS

SAP-K humanised heavy chain V region variant H2 nucleotide sequence (codon optimised) (SEQ ID NO:69)

[0223]

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAAGTGAAGAAGCCCGGCAGCAGCGTGAAAGTGAGCT
 GCAAGGCCAGCGGTACACCTTCACCAGCTACTGGATGCACTGGGTGAGGCAGGCACCCGGCCAG
 GGCCTGGAGTGGATCGGCATGATCCACCCCAACAGCGTGAACACCAACTACAACGAGAAGTTCAAG
 AGCAGAGCCACCATCACCGCCGACAAGAGCACCAGCACCGCCTATATGGAGCTGAGCTCTCTGAGG
 AGCGAGGATACCGCGTGTACTACTGCGCCAGGAGGAACGACTACTACTGGTACTTCGACGTCTGG
 GGCCAGGGCACACTAGTGACCGTGTCCAGC

SAP-K humanised heavy chain V region variant H2 amino acid sequence (SEQ ID NO:39)

[0224]

QVQLVQSGAEVKKPGSSVKVSKASGYTFTSYWMHWVRQAPGQGLEWIGMIHPNSVNTNYNEKFKS
 RATITADKSTSTAYMELSLRSEDVAVYVCARRNDYYWYFDVWGQGLTVTVSS

SAP-K humanised heavy chain V region variant H3 nucleotide sequence (codon optimised) (SEQ ID NO:70)

[0225]

CAGGTGCAGCTGGTGCAGAGCGGCGCCGAAGTGAAGAAGCCCGGCAGCAGCGTGAAAGTGAGCT
 GCAAGGCCAGCGGTACACCTTCACCAGCTACTGGATGCACTGGGTGAGGCAGGCACCCGGCCAG
 GGCCTGGAGTGGATCGGCATGATCCACCCCAACAGCGTGAACACCAACTACAACGAGAAGTTCAAG
 AGCAGAGCCACCTGACCGTGGACAAGAGCACCAGCACCGCCTATATGGAGCTGAGCTCTCTGAGG
 AGCGAGGATACCGCGTGTACTACTGCGCCAGGAGGAACGACTACTACTGGTACTTCGACGTCTGG
 GGCCAGGGCACACTAGTGACCGTGTCCAGC

SAP-K humanised heavy chain V region variant H3 amino acid sequence (SEQ ID NO:40)

[0226]

QVQLVQSGAEVKKPGSSVKVSKASGYTFTSYWMHWVRQAPGQGLEWIGMIHPNSVNTNYNEKFKS
RATLTVDKSTAYMELSSLRSEDTAVYYCARRNDYYWYFDVWGQGLTVTVSS

SAP-K humanised light chain V region variant L0 nucleotide sequence (codon optimised) (SEQ ID NO:71)

[0227]

GACATCCAGATGACCCAGAGCCCCTCTCACTGAGCGCTAGCGTGGGCGACAGGGTGACCAACACC
TGCAAGGCCAGCCAGAACGTGAACAGCAACGTGGCTGGTACCAGCAGAAGCCCGCAAAGCCCC
CAAGCTCCTGATCTACAGCGCCAGCTACAGATATAGCGCGTGCCTAGCAGTTTAGCGGCAGCGG
AAGCGGGACCGATTTCACCCCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACTTACTACTGC
CAGCAGTGCAACAACCTACCCCTTACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG

SAP-K humanised light chain V region variant L0 amino acid sequence (SEQ ID NO:41)

[0228]

DIQMTQSPSSLSASVGDRTITCKASQNVNSNVAWYQQKPGKAPKLLIYSASYRYSVPSRFSGSGSGTD
FTLTISLQPEDFATYYCQQCNYPFTFGQGTKLEIK

SAP-K humanised light chain V region variant L1 nucleotide sequence (codon optimised) (SEQ ID NO:72)

[0229]

GACATCCAGATGACCCAGAGCCCCTCTCACTGAGCGCTAGCGTGGGCGACAGGGTGACCAACACC
TGCAAGGCCAGCCAGAACGTGAACAGCAACGTGGCTGGTACCAGCAGAAGCCCGCAAAGCCCC
CAAGGCCCTGATCTACAGCGCCAGCTACAGATATAGCGCGTGCCTAGCAGTTTAGCGGCAGCGG
AAGCGGGACCGATTTCACCCCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACTTACTACTGC
CAGCAGTGCAACAACCTACCCCTTACCTTCGGCCAGGGCACCAAGCTGGAGATCAAG

SAP-K humanised light chain V region variant L1 amino acid sequence (SEQ ID NO:42)

[0230]

DIQMTQSPSSLSASVGDRTITCKASQNVNSNVAWYQQKPGKAPKALIIYSASYRYSVPSRFSGSGSGT
DFTLTISLQPEDFATYYCQQCNYPFTFGQGTKLEIK

SAP-K humanised H3 heavy chain nucleotide sequence (codon optimised) (SEQ ID NO:75)

[0231]

CAGGTGCAGCTGGTGAGAGCGGCCGAAGTGAAGAAGCCCGGCAGCAGCGTGAAAGTGAGCT
GCAAGGCCAGCGGTACACCTTACCAGCTACTGGATGCACTGGGTGAGGCAGGCACCCGCCAG
GGCCTGGAGTGGATCGGCATGATCCACCCCAACAGCGTGAACACCAACTACAACGAGAAGTTCAAG
AGCAGAGCCACCCTGACCGTGGACAAGAGCACCAGCACCGCCTATATGGAGCTGAGCTCTTGAGG
AGCGAGGATACCGCGTGTACTACTGCGCCAGGAGGAACGACTACTACTGGTACTTCGACGTCTGG
GGCCAGGGCACACTAGTGACCGTGTCCAGCGCCAGCACCAAGGGCCCCAGCGTGTCCCCCTGGCC

CCCAGCAGCAAGAGCACCAGCGGCGGCACAGCCGCCCTGGGCTGCCTGGTGAAGGACTACTTCCCC
 GAACCGGTGACCGTGTCTGGAACAGCGGAGCCCTGACCAGCGGCGTGACACCTTCCCCGCCGTG
 CTGCAGAGCAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCCAGCAGCAGCCTGGGCAC
 CCAGACCTACATCTGTAACGTGAACCAAGCCAGCAACACCAAGGTGGACAAGAAGGTGGAGCC
 CAAGAGCTGTGACAAGACCCACACCTGCCCCCTGCCCTGCCCCGAGCTGCTGGGAGGCCCCAG
 CGTGTTCCTGTTCCCCCAAGCCTAAGGACACCCTGATGATCAGCAGAACCCCGAGGTGACCTGT
 GTGGTGGTGATGTGAGCCACGAGGACCCTGAGGTGAAGTTCACTGGTACGTGGACGGCGTGGA
 GGTGCACAATGCCAAGACCAAGCCAGGAGGAGCAGTACAACAGCACCTACCGGGTGGTGCCG
 TGCTGACCGTGTGCACCAAGATTGGCTGAACGCAAGGAGTACAAGTGAAGGTGTCCAACAAGG
 CCCTGCCTGCCCTATCGAGAAAACCATCAGCAAGGCCAAGGGCCAGCCAGAGAGCCCCAGGTGT
 ACACCTGCCCTTAGCAGAGATGAGCTGACCAAGAACCAGGTGTCCCTGACCTGCCTGGTGAAGG
 GCTTCTACCCAGCGACATCGCCGTGGAGTGGGAGAGCAACGGCCAGCCGAGAACAATAACAAGA
 CCACCCCCCTGTGCTGGACAGCGATGGCAGCTTCTTCTGTACAGCAAGCTGACCGTGACAAGAG
 CAGATGGCAGCAGGGCAACGTGTTCACTGCTCCGTGATGCACGAGGCCCTGCACAATCACTACAC
 CCAGAAGAGCCTGAGCCTGTCCCCTGGCAAG

SAP-K humanised H3 heavy chain amino acid sequence (SEQ ID NO:76)

[0232]

QVQLVQSGAEVKKPGSSVKVSCASGYTFTSYWMHWVRQAPGQGLEWIGMIHPNSVNTNYNEFKS
 RATLTVDKSTSTAYMELSSLRSEDTAVYYCARRNDYYWYFDVWVGQTLTVSSASTKGPSVFPLAPSSKS
 TSGGTAALGCLVKDYFPEPTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK
 PSNTKVDKKVEPKSCDKHTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
 WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE
 PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

SAP-K humanised L0 light chain nucleotide sequence (codon optimised) (SEQ ID NO:77)

[0233]

GACATCCAGATGACCCAGAGCCCCTCTTCACTGAGCGCTAGCGTGGGCGACAGGGTGACCATCACC
 TGCAAGGCCAGCCAGAACGTGAACAGCAACGTGGCCTGGTACCAGCAGAAGCCCGGCAAAGCCCC
 CAAGCTCCTGATCTACAGCGCCAGCTACAGATATAGCGGCGTGCTAGCAGGTTTAGCGGCAGCGG
 AAGCGGGACCGATTTCACCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACTTACTACTGC
 CAGCAGTGAACAACCTACCCCTTACCTTCGGCCAGGGCACCAAGCTGGAGATCAAGCGTACGGTG
 GCCGCCCCCAGCGTGTTCATCTTCCCCCAGCGATGAGCAGCTGAAGAGCGGCACCGCCAGCGTG
 GTGTGTCTGCTGAACAACTTCTACCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAATGCCCTG
 CAGAGCGGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAAGGACTCCACCTACAGCCTGAG
 CAGCACCTGACCTGAGCAAGGCCGACTACGAGAAGCACAAGGTGTACGCTGTGAGGTGACCCA
 CCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGCGAGTGC

SAP-K humanised L0 light chain amino acid sequence (SEQ ID NO:78)

[0234]

DIQMTQSP5SLASVGDRTITCKASQNVNSNVAWYQQKPGKAPKLLIYSASYRYSRVPSRFSGSGSGTD
 FTLTISSLPEDFATYYCQQCNYPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
 AKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
 EC

Leader sequence for immunoglobulin chains (SEQ ID: 79)

[0235] MGWSCILFLVATATGVHS

Example 4: - Antibody expression

Recombinant antibody expression

[0236] Expression plasmids encoding the heavy and light chains respectively of chimeric or humanised antibodies were transiently co-transfected into HEK2936E cells by lipid transfection using Fectin 293. Cells were grown in Freestyle expression media 293 with 10% pluronic F68 and 50mg/ml geneticin, 37 degrees C, 5% CO₂ for 72 - 120 hrs, supernatant was harvested by centrifugation. In some instances the supernatant material was used as the test article in binding assays. In other instances, the supernatant material was filter sterilised and the antibody recovered by affinity chromatography using Protein A MAbSelect SuRE column followed by dialysis into PBS.

Hybridoma antibody expression

[0237] The hybridoma cells were grown in shake flasks in Ex620 medium supplemented with 4mM glutamax and 10% low IgG FCS. The cells were passaged and weaned off serum until growing well in serum free medium. The cells were then used as a seed for a 10L wavebag. The cells were grown in the wavebag at 22 rocks/min, 37 degrees C, 5% CO₂ @ 0.1L/min until viability dropped to 30%. The conditioned medium was collected by sterile filtration. Antibody was recovered by affinity chromatography using recombinant Protein A followed by dialysis into PBS.

Examples 5-7: Comparative data between hybridomas and/or chimeric mAbs and/or humanised Mabs

Example 5: Comparison of SAP-K and SAP-E hybridomas in human SAP binding ELISA

[0238] 1 µg/mL or 5 µg/mL human SAP was directly immobilised onto an ELISA plate and blocked with 1%BSA/TBS plus 0.05% TWEEN20. Anti-SAP antibodies from purified material were titrated across the plate. Bound antibody was detected by treatment with a horse-radish peroxidase (HRP) -conjugated rabbit-anti-mouse IgG antibody (Dako, P0260). The ELISA was developed using O-phenylenediamine dihydrochloride (OPD) peroxidase substrate (Sigma, P9187).

[0239] Figure 1 shows the binding curves for murine antibodies SAP-E and SAP-K at a 1 µg/mL coating concentration of human SAP.

[0240] Figure 2 shows the binding curves for murine antibodies SAP-E and SAP-K at a 5 µg/mL coating concentration of human SAP.

[0241] At the 5 µg/mL coating concentration, SAP-K and SAP-E showed similar binding to the immobilised human SAP, whereas at the 1 µg/mL lower density coating SAP-K showed greater binding than the SAP-E. All subsequent human SAP binding ELISAs using this format used the lower density 1 µg/mL coating concentration to distinguish between the binding properties of the two antibodies.

Example 6: Comparison of SAP-K and SAP-E chimeric/humanised mAbs in human SAP binding ELISA

[0242] 1µg/mL human SAP was directly immobilised onto an ELISA plate and blocked with 1%BSA/TBS plus 0.05% TWEEN20. Anti-SAP antibodies from the test supernatants or purified material were titrated across the plate. Bound antibody was detected by treatment with goat anti-human Kappa Light Chains peroxidase conjugate (Sigma, A7164). The ELISA was developed using O-phenylenediamine dihydrochloride (OPD) peroxidase substrate (Sigma, P9187).

[0243] Figure 3 shows the binding curves for chimeric antibodies cSAP-E and cSAP-K. The profile of the curves for the chimeric antibodies is the same as that of the equivalent hybridomas.

[0244] Figure 4 shows the binding curves for SAP-K H0L0, SAP-K H1L0, SAP-K H2L0 and SAP-K H3L0 compared to the SAP-K chimera and the SAP-E H1L1 compared to the SAP-E chimera. An irrelevant human IgG1 kappa antibody was also tested as a negative control. The data shows that humanisation of the SAP-K antibody resulted in a loss of human SAP binding activity of approximately 2-fold compared to the parental SAP-K chimera, whilst the humanised SAP-E antibody retained binding activity compared to the parental SAP-E chimera.

Example 7 Competition ELISA

[0245] ELISA plates were coated with human SAP at either 1µg/mL (for competition with SAP-K chimera) or 5µg/mL (for competition with SAP-E chimera) and blocked with 1% BSA/PBS. A constant concentration of chimeric anti-SAP mAb was mixed with serial diluted (1:1) amounts of mouse anti-SAP mAbs. Plates were washed and the amount of chimeric antibody bound to the immobilised human SAP was detected using goat anti-human Kappa Light chain peroxidase conjugate (Sigma, A7164). The ELISA was developed using O-phenylenediamine dihydrochloride (OPD) peroxidase substrate (Sigma, P9187).

[0246] Figure 5 shows purified SAP-K and SAP-E murine monoclonal antibodies in the competition ELISA with the SAP-E chimera.

[0247] Figure 6 shows purified SAP-K and SAP-E murine monoclonal antibodies in the competition ELISA with the SAP-K chimera.

[0248] In both figures 5 and 6 no competition is observed between the SAP-E and SAP-K antibodies showing that the two antibodies bind to distinct epitopes on the human SAP molecule.

Example 8: Determination of kinetics of binding

Biacore analysis of binding of humanised anti-SAP antibody variants to purified human and purified cynomolgus monkey SAP.

[0249] Human and cynomolgus monkey SAP were immobilised on a Biacore C1 chip by primary amine coupling in accordance with the manufacturer's instructions. Humanised anti-SAP antibody contained in culture supernatants and purified chimeric antibodies at 512nM were passed over both human and cynomolgus monkey SAP surfaces and binding sensograms obtained. All runs were double referenced with a buffer injection for purified sample or media for the supernatant samples over the human and cyno SAP surfaces. Analysis was carried out at 25°C using HBS-EP buffer. Regeneration of surface was done in the presence of 3M MgCl₂ and did not affect the ability of antibodies to rebind to human SAP in a subsequent cycle. Data were analysed using the 1 to 1 dissociation model within the Biacore T100 evaluation software.

[0250] The data generated in Tables 6a and 6b show off-rates (kd) of the humanised SAP-E and SAP-K antibody supernatants respectively. The values were based on a single curve and used for ranking purposes between the different constructs for binding to human SAP. Humanised SAP-E antibodies showed better off-rates than the humanised SAP-K antibodies for binding human SAP. A number of the SAP-K humanised antibody variants showed binding to cynomolgus monkey SAP (N.B. the SAP-K chimera bound cynomolgus monkey SAP) whilst none of the humanised SAP-E antibody variants bound cynomolgus monkey SAP (N.B. the SAP-E chimera likewise did not bind cynomolgous monkey SAP). Humanised SAP-E variants which contained either the straight graft humanised heavy chain (H0) or the straight graft humanised light chain (L0) or a combination of both showed the poorest off-rates. The SAP-E humanised L1 light chain was the best light chain variant and combination of the L1 with

the H1 heavy chain variant gave a humanised antibody with an acceptable off-rate whilst keeping the number of back mutations to a minimum. Off-rate ranking of the humanised SAP-K variants showed the L0 straight graft to be the best humanised light chain variant and the H0 straight graft to be the poorest humanised heavy chain variant.

Table 6a

SAP-E Variant	Kd for human SAP (s ⁻¹)
SAP-E chimera	3.83E-03
SAP-E H1L1	4.80E-03
SAP-E H4L1	5.43E-03
SAP-E H1L2	5.51E-03
SAP-E H3L1	5.76E-03
SAP-E H4L2	5.80E-03
SAP-E H2L1	6.09E-03
SAP-E H3L2	6.31E-03
SAP-E H2L2	6.52E-03
SAP-E H1L0	8.09E-03
SAP-E H3L0	9.10E-03
SAP-E H2L0	9.79E-03
SAP-E H4L0	9.81E-03
SAP-E H0L1	4.02E-02
SAP-E H0L2	4.29E-02
SAP-E H0L0	5.35E-02

Table 6b

N.B. Kd is for human SAP		
	kd (s ⁻¹)	Binding to cyno SAP
SAP-K chimera	6.64E-03	Yes
SAP-KH1L0	1.71E-02	poor
SAP-K H3L0	1.84E-02	Yes
SAP-K H2L0	2.04E-02	Yes
SAP-K H3L1	2.36E-02	yes
SAP-K H0L0	2.63E-02	no
SAP-K H1L1	2.96E-02	poor
SAP-K H2L1	3.21E-02	poor
SAP-K H0L1	4.79E-02	no

Biacore analysis of binding of anti-SAP antibodies to purified human SAP directly immobilised on a solid phase support

[0251] Human SAP was immobilised on a Biacore CM3 chip by primary amine coupling in accordance with the manufacturer's instructions. Anti SAP antibodies were passed over this surface at 512, 128, 32, 8, 2, 0.5nM and binding sensorgrams obtained. All runs were double referenced with a buffer injection over the human SAP surface. Analysis was carried out at 25°C using HBS-EP buffer. Regeneration of surface was done by allowing buffer to flow over the surface for several minutes and did not affect the ability of human SAP to rebind antibodies in a subsequent cycle. Data were analysed from the 128 - 0.5nM runs using the bivalent analyte model inherent to the Biacore T100 evaluation software.

[0252] The data generated and compiled in table 7 were meant for comparison between the constructs and show that SAP-K antibodies have a better association rate in this assay while SAP-E antibodies show better dissociation rates. Furthermore,

humanization had not altered the binding kinetics of SAP-E antibody whilst for SAP-K a loss in association and dissociation rate was observed following humanisation.

Table 7

	Ka (M ⁻¹ .s ⁻¹)	Kd (s ⁻¹)	KD (nM)
SAP-K chimera	4.06E+5	7.59E-03	18.7
SAP-K H0L0	6.08E+4	4.49E-02	739
SAP-K H1L0	1.15E+5	1.78E-02	155
SAP-K H2L0	1.15E+5	2.20E-02	191
SAP-K H3L0	1.50E+5	1.92E-02	128
SAP-E chimera	2.64E+4	2.18E-03	82.6
SAP-E H1L1	2.64E+4	2.07E-03	78.3

Biacore analysis of binding of anti-SAP antibodies to purified human SAP captured on immobilised O-phosphoethanolamine

[0253] O-phosphoethanolamine was immobilised on a Biacore CM5 chip by primary amine coupling in accordance with the manufacturer's instructions. Human SAP was then captured on the surface in the presence of calcium chloride, in order to replicate in the Biacore system *in vitro*, the precise orientation of SAP molecules bound to amyloid fibrils *in vivo*. Anti SAP antibodies were then passed over this surface at 256, 64, 16, 4, 1nM and a binding sensorgrams obtained. Analysis was carried out at 25°C using 4% BSA, 10mM Tris, 140mM NaCl, 2mM CaCl₂, 0.05% surfactant P20, 0.02% NaN₃, pH 8.0 as running buffer. Regeneration was achieved using two pulses of Tris-EDTA (10mM Tris, 140mM NaCl, 10mM EDTA, pH 8.0) which removed the bound human SAP but did not significantly affect subsequent binding of SAP to the immobilised phosphoethanolamine. Data generated were double referenced with a buffer injection over the human SAP surface and analyzed using the bivalent analyte model in the Biacore T100 evaluation software.

[0254] The data generated, as shown in Table 8, are intended only for comparison between the constructs. They do not constitute accurate kinetic values, due to possible modification of binding by the avidity effect inherent in the assay format. Avidity is more likely to have affected antibody dissociation rates, leading to lower calculated KD values. Furthermore, for all the SAP-E antibodies, the dissociation rate (kd) obtained is outside the limit of the Biacore measurement range. Nevertheless, the results indicate tight binding of the anti-SAP antibodies to human SAP immobilised by interaction of the SAP with a solid phase ligand, just as it is in amyloid deposits *in vivo*, which is the therapeutic target of the present invention.

Table 8

	ka (M ⁻¹ .s ⁻¹)	kd (s ⁻¹)	KD (nM)
SAP-K chimera	3.32E+5	2.97E-4	0.895
SAP-E chimera	2.03 E+4	9.12E-7	4.49E-11
Mouse SAP-K	3.00E+5	2.19E-4	0.730
Mouse SAP-E	3.15E+4	1.51E-8	4.79E-13
SAP-K H3L0	1.36E+5	5.01E-3	36.8
SAP-E H1L1	1.94E+4	1.58E-7	8.14E-12

Example 9: Amino acid scan at position 91 of SAP-K L0 humanised light chain

[0255] Site-directed saturation mutagenesis was used to generate a panel of variants where the cysteine residue at position 91 (Kabat numbering) was potentially substituted with all other 19 amino acids in a single reaction by using a mutagenesis primer encoding NNK at this position (where N codes for adenosine or cytidine or guanosine or thymidine and K codes for guanosine or thymidine). From Biacore off-rate ranking carried out on antibody supernatant for the variants generated, four were selected for scale up in the HEK2936E cells and purification. Biacore kinetic analysis using the O-phosphoethanolamine method as detailed in Example 7 showed that the variant with alanine at position 91 (SEQ ID NO:43) had an improved affinity compared to

the wild-type; KD values of 0.436 nM and 36.8 nM were measured respectively. N.B. all variants were tested in the same experiment used to produce the results shown in table 7.

[0256] Other variants, for example glycine, serine and valine improved binding with respect to H3L0, but to a lesser extent than alanine. In addition, the fact that these four variants had better binding properties than L0 was also observed in a binding ELISA and a Biacore off-rate ranking experiment when the light chains were paired with H1.

SAP-K humanised light chain V region variant L0 91A nucleotide sequence (codon optimised) (SEQ ID NO:73)

[0257]

GACATCCAGATGACCCAGAGCCCTCTTCACTGAGCGCTAGCGTGGGCGACAGGGTGACCATCACC
TGCAAGGCCAGCCAGAACGTGAACAGCAACGTGGCCTGGTACCAGCAGAAGCCCGGCAAAGCCCC
CAAGCTCCTGATCTACAGCGCCAGCTACAGATATAGCGCGTGCCTAGCAGTTTAGCGGCAGCGG
AAGCGGGACCGATTTCACCTGACCATCAGCAGCCTGCAGCCCGAGGACTTCGCCACTTACTACTGC
CAGCAGGCGAACAACCTACCCCTTACCTTCGCCAGGGCACCAAGCTGGAGATCAAG

SAP-K humanised light chain V region variant L0 91A amino acid sequence (SEQ ID NO:74)

[0258]

DIQMTQSPSSLSASVGDRTITCKASQNVNSNVAWYQQKPGKAPKLLIYSASYRYSVPSRFSGSGSGTD
FTLTISLQPEDFATYYCQQANNYPFTFGQGTKLEIK

Example 10: Complement dependence of amyloid clearance by anti-SAP antibody.

[0259] The role of complement in amyloid clearance by anti-SAP antibody was investigated by comparing the efficiency of the treatment between mice with complement deficiency and normal, complement sufficient, animals. Targeted deletion of the gene for C1q blocks activation of the classical complement pathway, which is initiated by binding of C1q to antibody-antigen complexes, but C3 activation, the pivotal functional step responsible for chemotaxis and opsonisation, the major biological functions of complement, can still proceed via the alternative and lectin pathways as well as by direct C3 cleavage by non-complement serine proteinases. Targeted deletion of the gene for C3 completely abrogates these functions.

Induction of AA amyloidosis

[0260] AA amyloidosis was induced and confirmed in two groups of complement deficient mice: C3 knockouts (n=14) and C1q knockouts (n=12), and in 15 wild-type mice. All mice were pure line C57BL/6. Each mouse received a single dose of amyloid enhancing factor, an extract of amyloidotic tissue containing amyloid fibrils (Baltz et al, (1986) Plenum Press, New York, pp. 115-121), by intravenous injection followed 4 days later by 10 daily subcutaneous injections of 10% w/v casein in solution in 0.1M NaHCO₃ administered over a 12 day period (Botto et al, (1997) Nature Med., 3: 855-859). Casein elicits persistent acute inflammation and a sustained increase in serum amyloid A protein (SAA) production leading to AA amyloid deposition in all animals. Seven days after the last casein injection, KI was introduced into the drinking water of all mice and 3 days later each mouse received an intravenous injection of a standard dose of ¹²⁵I-labelled human SAP (Hawkins et al, (1990) J. Clin. Invest., 86: 1862-1869 and Hawkins et al, (1988) J. Exp. Med., 167: 903-913). All mice underwent whole body counting 24h and 48h after the tracer injection to determine retention of radioactivity, a precise index of whole body amyloid load. Ten days after the ¹²⁵I-SAP tracer injection, all mice were 'loaded' with human SAP by a single intraperitoneal injection of 10 mg per mouse of isolated pure human SAP. Human SAP injected into amyloidotic mice localises in the amyloid deposits and persists there with a half life of about 3-4 days whilst any human SAP not bound to amyloid is cleared from the circulation with a half life of about 3-4 hours (Hawkins et al, (1988) J. Exp. Med., 167: 903-913 and Pepys et al, (2002) Nature, 417: 254-259).

[0261] Immunohistochemical staining with anti-human SAP antibody in spleen of an amyloidotic mouse after injection of isolated pure human SAP shows that there is strong positive staining of all the amyloid deposits in their typical marginal zone distribution.

This bound human SAP is the target of the therapeutic anti-SAP antibody according to the present invention.

Anti-SAP treatment

[0262] Three days after the human SAP injection, when human SAP was no longer detectable in the circulation, all mice except two in each of the complement knockout groups received a single intraperitoneal injection of 1 ml of the whole IgG fraction (batch no. 2866) of monospecific sheep anti-human SAP antiserum at 50 mg/ml in solution in phosphate buffered saline (PBS), containing 7 mg/ml of actual anti-SAP antibody. The antiserum was produced by The Binding Site Ltd, Birmingham, UK, using human SAP (rigorously purified to 100% in the University College London Centre for Amyloidosis and Acute Phase Proteins) and proprietary immunisation procedures. All animals were then killed 15 days after anti-SAP administration for histological estimation of amyloid load by alkaline alcoholic Congo red staining (Puchtler, H., Sweat, F. and Levine, M. (1962) On the binding of Congo red by amyloid. *J. Histochem. Cytochem.*, 10: 355-364). Congo red sections of spleen and liver of all animals were independently examined by one or more expert observers, blinded to the treatment each mouse had received, and scored for the amount of amyloid present as previously reported (Botto et al, (1997) *Nature Med.*, 3 : 855-859). The scores of 1-5 represent an approximately log base 10 ranking scale from 1, corresponding to one or two tiny specks of amyloid among several sections of a particular organ, to 5, corresponding to abundant widespread deposits comprising about 10,000 times more amyloid than grade 1 (Botto et al, (1997) *Nature Med.*, 3: 855-859). The scores of the different observers were always highly concordant although some observers also used intermediate integer.5 scores. The arithmetic mean of the scores of all observers for each organ in each animal were used for statistical analysis.

Results

[0263] In marked contrast to the effective clearance of amyloid deposits in the complement sufficient wild-type mice, there was still abundant amyloid present in both groups of complement deficient animals although it tended to have a more fragmented appearance than in the two control complement deficient mice of each type. The median, range, spleen amyloid scores were: wild type, 1.17, 0.0-1.5, n=15; C3 knockout, 1.92, 1.17-4.33, n=12; C1q knockout, 1.25, 1.17-3.5, n=10 (Kruskal-Wallis non-parametric ANOVA, $P<0.001$). The differences between the wild type controls and both complement deficient groups were significant, $P<0.001$ for the C3 knockouts and $P=0.036$ (with Bonferroni correction for multiple comparisons) for the C1q knockouts, but the difference between the C3 and C1q knockouts was not significant, $P=0.314$ (Mann-Whitney U tests).

Discussion

[0264] In mice lacking either C1q or C3, anti-SAP treatment did not clear amyloid deposits as effectively as in complement sufficient wild-type mice. The therapeutic efficacy of anti-SAP is thus very substantially complement dependent and is not mediated by IgG antibody binding alone which could, in theory, engage phagocytic cells via their Fc(γ) receptors. Nevertheless the more fragmented appearance of the persistent amyloid deposits in the complement deficient mice suggested at least some effect of antibody alone. Also the trend to more clearance in C1q deficient compared to C3 deficient animals suggested that C3 activation is critical and that some complement activation may be taking place in the absence of C1q.

Example 11: Requirement for intact IgG anti-SAP antibody

[0265] Complement activation by IgG antibody requires the whole intact molecule, including the Fc region, and proceeds via the classical pathway initiated by binding of C1q. However, in some antibody-antigen systems, complement activation via the alternative pathway can be mediated by the F(ab)₂ fragment. In order to confirm the complement dependence of amyloid clearing by anti-SAP antibody and to investigate the potential requirement for the Fc region of the antibody, the effect was tested of F(ab)₂ anti-SAP antibody which was produced by pepsin cleavage at pH 4.0 of the IgG fraction of the sheep polyclonal anti-human SAP antiserum (batch 2866) and purified by standard methods.

Induction and treatment of AA amyloidosis

[0266] AA amyloidosis was induced and confirmed in wild-type C57BL/6 mice as detailed in Example 10 above. After loading the amyloid deposits with human SAP also as detailed in Example 10, groups of mice were treated with whole IgG fraction of the sheep polyclonal anti-human SAP antiserum, with buffer vehicle alone or with the F(ab)₂ fragment of the IgG fraction. The dose of anti-SAP antibody activity injected was 7.28 mg per mouse receiving F(ab)₂ and 7 mg (50 mg of total IgG as usual) per mouse receiving whole IgG. All mice were killed 14 days later for estimation of amyloid load by Congo red staining.

Results

[0267] Clearance of amyloid deposits was almost complete in mice receiving IgG anti-SAP antibody compared to the massive amyloid deposits in the control mice receiving vehicle alone. The mice receiving F(ab)₂ had less amyloid than untreated controls, but still substantially more than the mice treated with whole IgG anti-SAP antibody (Table 9).

Table 9. Reduced efficacy of F(ab)₂ anti-SAP compared to intact IgG antibody in clearing amyloid deposits.

Group (treatment, group size)	Amyloid score median, range	
	Spleen	Liver
1 (no antibody, n=10)	4.0, 4.0-4.33	3.5, 2.67-4.67
2 (IgG anti-SAP antibody, n=8)	1.0, 1.0-3.67*	1.25, 1.0-1.5
3 (F(ab) ₂ anti-SAP antibody, n=5)	2.17, 1.33-3.0	1.67, 1.33-1.67

Kruskal-Wallis test: spleen, P<0.001; liver P<0.001
Mann-Whitney tests**: 1 vs 2, spleen & liver both, P<0.001; 1 vs 3, spleen & liver both, P=0.001; 2 vs 3, spleen, P=0.284; liver, P=0.019
*Single outlier in group 2 with heavy spleen amyloid despite IgG anti-SAP treatment. Excluding this animal gives a highly significant difference between efficacy of IgG and F(ab)₂ anti-SAP antibody treatment. **Due to the multiple comparisons, a P value of 0.01 or less is required for significance

Discussion

[0268] The molar dose of F(ab)₂ anti-SAP antibody used in this study was about one third greater than that of IgG antibody, due to the smaller molecular weight of the F(ab)₂ fragment compared to whole IgG. For optimal effect on amyloid clearance the Fc is required. This is not because of direct involvement of cellular recognition by Fc(γ) receptors since the whole IgG was even less effective in complement deficient mice than was F(ab)₂ in complement sufficient, mice. It is likely that the high dose of F(ab)₂ that was administered was able to activate some complement via the alternative pathway.

Example 12: Requirement for macrophages

[0269] The histological and histochemical studies described in US 2009/0191196 show that the cells which infiltrate, surround and phagocytose the amyloid deposits in mice treated with anti-SAP antibody are macrophages. In order to confirm that macrophages are indeed responsible for the clearance of the amyloid, the effect of treatment with the whole IgG fraction of the sheep polyclonal anti-human SAP antiserum (batch 2866) was tested in mice in which all macrophage activity had been inhibited by administration of liposomal clodronate. The reagents, experimental protocol and effects on macrophage function of liposomal clodronate are well established and extensively documented (Van Rooijen et al, (2002) J. Liposome Research. Vol. 12. Pp, 81-94).

Induction and treatment of AA amyloidosis

[0270] After induction and confirmation of AA amyloidosis in wild-type mice, using the protocol detailed in Example 10 above, all animals received a single intraperitoneal dose of 10 mg of isolated pure human SAP to load their deposits with human SAP. The test group then received 0.3 ml of liposomal clodronate intraperitoneally immediately and on days 2, 7 and 14 thereafter. One control group and the test group received a single intraperitoneal dose of 50 mg of the IgG fraction of sheep anti-human SAP antiserum on day 3 after the human SAP injection. A second control group received no anti-SAP and no other additional

treatment. All mice were killed for estimation of amyloid load by Congo red staining 14 days after administration of the anti-SAP to the test and antibody control groups.

Results

[0271] Treatment with anti-SAP produced almost complete clearance of amyloid deposits compared to the group which received no antibody. In contrast, in mice which received the liposomal clodronate in a regime known to completely ablate macrophage function, there was no clearance of amyloid deposits (Table 10).

Table 10. Macrophage depletion inhibits clearance of amyloid deposits by anti-SAP antibody.

Group (treatment, group size)	amyloid score median, range	
	Spleen	Liver
1 (clodronate plus anti-SAP, n=13)	4.83, 2.0-5.0	3.17, 2.0-3.5
2 (anti-SAP only, n=12)	1.33, 0.67-3.5	1.0, 0.67-2.5
3 (none, n=12)	4.0, 3.5-4.5	2.83, 1.0-3.17
Kruskal-Wallis test: spleen, $P < 0.001$; liver $P < 0.001$		
Mann-Whitney tests with Bonferroni correction: 1 vs 2: spleen & liver both, $P < 0.003$; 1 vs 3: spleen, $P = 0.078$; liver, $P = 0.411$; 2 vs 3, spleen & liver both, $P < 0.003$.		

Discussion

[0272] The result in this particular experiment confirmed that macrophage function is required for clearance of amyloid deposits by anti-human SAP antibody.

Example 13: Efficacy of mouse monoclonal anti-human SAP antibody, SAP-E, in clearing mouse systemic AA amyloid deposits.

[0273] The capacity of various monoclonal antibodies to mediate clearance of murine AA amyloid deposits containing human SAP was sought in comparison with the standard sheep polyclonal anti-human SAP antibody as a positive control.

Induction of AA amyloidosis and treatment

[0274] SAP knockout C57BL/6 mice transgenic for human SAP were created by crossing pure line C57BL/6 animals in which the mouse SAP gene has been deleted (Botto et al, (1997) Nature Med., 3: 855-859) with C57BL/6 mice bearing a human SAP transgene (Yamamura et al, (1993) Mol. Reprod. Dev., 36: 248-250 and Gillmore et al, (2004) Immunology, 112: 255-264). These mice thus lack mouse SAP but express human SAP at concentrations significantly greater than those seen in man. Systemic AA amyloidosis was induced in the human SAP transgenic mouse SAP knockout mice as described in Example 10, and 9 days after the final injection of casein into the mice, the presence and extent of amyloid deposition were confirmed as usual by whole body counting of amyloid after injection of a tracer dose of 125 I-labelled human SAP. All mice had substantial and comparable amounts of amyloid, and were allocated into closely matched groups to receive the different treatments. One week after the tracer injection, each mouse received a single dose of 5 mg CPHPC by intraperitoneal injection, to deplete their circulating human SAP, followed 5h later via the same route by either the standard sheep polyclonal anti-human SAP IgG fraction (batch 2866, 1 ml at 50 mg/ml total protein containing 7 mg/ml anti-human SAP antibody) or 5 mg of one of nine different isolated pure monoclonal anti-human SAP antibodies (Table 11). All mice were killed 21 days after the antibody injection and amyloid load was determined by Congo red histology of their spleens.

Table 11. The presence of amyloid in spleen of mice with systemic AA amyloidosis after treatment with CPHPC and various anti-human SAP antibodies.

Antibody treatment	Antibody isotype	Amyloid score median, range
none		3,3-5

Antibody treatment	Antibody isotype	Amyloid score median, range
polyclonal	NA	1,1-1
monoclonal SAP-A	IgG1	3, 2-4
monoclonal SAP-B	IgG2a	3, 2-4
monoclonal SAP-C	IgG1	4,2-4
monoclonal SAP-D (n=1)	IgG1	4
monoclonal SAP-E	IgG2a	1, 1-1
monoclonal SAP-F (n=1)	IgG1	2
monoclonal SAP-G	IgG1	3, 2-4

[0275] Among the monoclonal antibodies tested, only SAP-E produced clearance of the amyloid deposits but its effect was the same as the highly reproducible and dramatic action of the sheep polyclonal antibody. Importantly SAP-E is of the mouse IgG2a isotype which is known to activate mouse complement while all the other monoclonals except SAP-B were mouse IgG1 isotype which is not complement activating. Although SAP-B is a mouse IgG2a isotype, its binding to SAP *in vitro* was notably less than that of SAP-E and evidently was not sufficient *in vivo* to be effective.

Discussion

[0276] These results demonstrate that a sufficiently avid, complement activating, IgG2a mouse monoclonal anti-human SAP antibody mediates amyloid clearance *in vivo* as effectively as sheep polyclonal anti-human SAP antibody.

Example 14: Comparative characterisation of Monoclonal mouse anti-human SAP antibodies, SAP-K and SAP-E, *in vitro*.

[0277] SAP-K was selected from among the 6 different, most avidly binding, mouse IgG2a monoclonals, derived by standard techniques from immunization with purified human SAP and a conventional fusion to produce hybridomas which were cloned by routine methods. Among these IgG2a antibodies, SAP-K showed the greatest binding to immobilised human SAP. This was the case regardless of whether the human SAP had been directly immobilised on plastic surfaces by non-specific adherence or by covalent attachment, or by the specific calcium dependent binding of SAP to immobilised ligands, whether amyloid fibrils or the small molecule ligand, phosphoethanolamine. SAP-K also bound well to directly immobilised SAP in the presence or absence of calcium, and if the SAP had previously been complexed with CPHPC and then covalently 'fixed' in the decameric SAP-CPHPC complex (Pepys, M.B. et al (2002) Targeted pharmacological depletion of serum amyloid P component for treatment of human amyloidosis. *Nature*, 417: 254-259; Kolstoe, S.E. et al (2009) Molecular dissection of Alzheimer's disease neuropathology by depletion of serum amyloid P component. *Proc. Natl. Acad. Sci. USA*, 106: 7619-7623). SAP-E also bound well to human SAP in all these different configurations. However the two antibodies differ significantly in that much more SAP-K than SAP-E became bound when human SAP was only sparsely available, for example when plates were exposed to just 1 µg/ml of human SAP for coating, whereas when there was more abundant immobilised SAP, for example when the coating solution contained 100 µg/ml of SAP, then there was more binding of SAP-E than SAP-K. This difference suggest that SAP-E binds optimally when more than one SAP molecule lies closely associated with another whilst SAP-K binds avidly to single isolated SAP molecules. This mechanism is supported by the finding that when human SAP was immobilised by capture on plates coated with polyclonal sheep anti-human SAP (batch 2866), which provides pairs of SAP molecules held closely together in the two arms of each sheep IgG antibody molecule, SAP-E bound better than SAP-K at all levels of human SAP input (Figure 7).

[0278] Figure 7 shows immunoradiometric assay for binding of monoclonal mouse antibodies to human SAP captured by immobilised sheep polyclonal anti-human SAP antibody. Substantially more SAP-E than SAP-K bound at all concentrations of human SAP offered. Each point is the mean of 3 replicates.

[0279] Very importantly, both SAP-E and SAP-K bound apparently equally well to native human SAP, shown by the similar immunoprecipitation of both antibodies in double immunodiffusion in agarose gel against both isolated pure human SAP and whole human serum. The similar binding of these two mouse monoclonal antibodies was reflected in the similar parameters measured in the Biacore instrument (BiacoreX, Pharmacia Biosensor AB, Uppsala, Sweden) using human SAP covalently immobilised on the chip (Table 12).

Table 12. Affinity of monoclonal antibodies for human SAP determined by Biacore

	k_a (M ⁻¹ sec ⁻¹)	k_d (sec ⁻¹)	K_D (M)
SAP-E	$2 \pm 5 \times 10^4$	$6 \pm 4 \times 10^{-5}$	$5 \pm 4 \times 10^{-9}$
SAP-K	$3.18 \pm 5 \times 10^4$	$1.7 \pm 0.9 \times 10^{-5}$	$1 \pm 1.7 \times 10^{-9}$
Values shown are mean and SD of 3 replicate measurements			

[0280] In contrast, although both antibodies bound to native human SAP in western blotting after agarose gel electrophoresis in physiological buffers, only SAP-E bound to human SAP in western blotting from reduced SDS-PAGE. SAP-E thus recognises denatured human SAP while SAP-K only recognises native human SAP and must be binding to a conformational epitope.

[0281] CNBr digestion of human SAP results in cleavage between 159M and 160W resulting in a new peptide where position 159 has been converted from methionine to homoserine lactone (termed 150-158-homoserine lactone). In western blotting from SDS-PAGE, SAP-E bound to the N-terminal 1-158-homoserine lactone polypeptide released by CNBr cleavage of SAP at residue Met159, but scarcely reacted with the 1-140 fragment released by chymotrypsin digestion in the absence of calcium (Figure 8). The epitope recognised by SAP-E must therefore be in the region 140-158 which evidently comprises some denaturation resistant secondary structure since SAP-E binding is not potently inhibited by the peptides 136-147, 138-149, 140-151 and 112-119 in solution. This is consistent with the kinetic stability and resistance to denaturation of SAP (Manning, M. and Colón, W. (2004) *Biochemistry*, 43: 11248-11254).

[0282] Figure 8 shows epitope mapping for monoclonal anti-human SAP antibody, SAP-E. A, complete amino acid sequence of human SAP showing the points at which it is cleaved by CNBr in 70%TFA (residue 159M) and by chymotrypsin, without reduction/carbamidomethylation, in ammonium bicarbonate in the absence of calcium, (residues 140Y and 144F). B, SDS-PAGE analysis of SAP cleaved with CNBr. Left panel: Coomassie blue stain; lane 1, untreated control SAP; lane 2, SAP after CNBr cleavage, showing trace residual uncleaved intact protomer and the expected fragments at approximately 20kD (residues 1-158-homoserine-lactone) and 5kD (160-204) respectively. These were precisely confirmed by mass spectrometry. Right panel: Western blot with SAP-5 showing intense staining of intact untreated SAP in lanes 1 (100 ng loaded) and 2 (10 ng), and also residual intact SAP and the larger residue 1-158-homoserine-lactone fragment in CNBr cleaved SAP in lanes 3 (600 ng), 4 (130 ng) and 5 (64 ng). Lane 6 contained isolated pure human CRP with which the SAP-5 did not react at all. C, SDS-PAGE analysis of SAP digested with chymotrypsin. Left panel: Coomassie blue stain; lane 1, untreated control SAP; lane 2, SAP after chymotrypsin digestion, showing the expected major fragments corresponding to residues 1-140 and 145-204. These were precisely confirmed by mass spectrometry. Right panel: Western blot with SAP-E showing intense staining of intact untreated SAP in lanes 1 (500 ng loaded) and 2 (100 ng), and also residual intact SAP in lanes 3 and 4 which contained the chymotrypsin digested SAP at different loadings. Very weak binding of SAP-E to the residue 1-140 fragment is seen only in lane 3 which was most heavily loaded. Lanes 5 (500 ng) and 6 (100 ng) contained isolated pure human CRP with which the SAP-E did not react at all. D, Sequence comparison between human SAP (h) and mouse SAP (m) for residues 136-147. Top panel, differences indicated above by residues shown in black in the murine sequence. Bottom panel, position of this extended loop with 140Y at its apex shown in white in the 3D subunit structure of human SAP. The different residues in the murine sequence are shown in black. The grey spheres represent the calcium atoms bound in the ligand binding pocket.

[0283] The conformational epitope recognised by SAP-K was identified by CLIPS® technology epitope mapping (Pepscan Presto BV) as the exposed peripheral loop, residues 121-131, at the circumference of the disc like pentameric native SAP molecule.

[0284] Figure 9 shows the location of the epitopes on human SAP recognised by SAP-K (A, highlighted in black, as determined by CLIPS® technology) and SAP-E (B, shown in white, 140-158 as determined by binding results with the CNBr cleavage product of SAP and the fragment released by chymotrypsin digestion in the absence of calcium).

Example 15: Efficacy of SAP-K mouse monoclonal anti-human SAP antibody in clearing amyloid deposits *in vivo* in the mouse AA amyloidosis model.

[0285] The potency of SAP-K was compared with the action of the standard sheep polyclonal antibody in clearing established systemic AA amyloid deposits in mice.

Induction of AA amyloidosis and treatment

[0286] AA amyloidosis was induced and confirmed in wild-type C57BL/6 mice as detailed in Example 10 above. After loading the amyloid deposits with human SAP also detailed in Example 10, groups of mice were treated with 50 mg per mouse of total IgG as the whole IgG fraction (batch 2866) of the sheep polyclonal anti-human SAP antiserum providing a dose of 7 mg of actual anti-SAP antibody, isolated purified SAP-K at a dose of 5 mg per mouse, isolated purified SAP-K at a dose of 1 mg per mouse, and, as a negative control, isolated purified monoclonal mouse IgG2a antibody specific for an unrelated human antigen and unreactive with either human SAP or any murine antigen. All mice were killed 17 days later for estimation of amyloid load by Congo red staining.

Results

[0287] The mice treated with 5 mg of SAP-K showed the same remarkable clearance of splenic and hepatic amyloid deposits as seen with the 7 mg dose of sheep polyclonal antibody. Only trace specks of amyloid remained in the spleens of the treated mice and none at all was detected in many of the livers, contrasting sharply with the extensive splenic and hepatic amyloid deposits in all animals which received the irrelevant control mouse IgG2a antibody (Table 13). At the lower doses of 1 mg, 0.5 mg and 0.1 mg (data not shown for 0.5 mg and 0.1 mg) of SAP-K per mouse, there was no significant effect.

Table 13. Effect of monoclonal mouse IgG2a anti-human SAP antibody SAP-K on visceral amyloid deposits in mice with systemic AA amyloidosis.

Group (treatment, group size)	Amyloid score	
	median, range	
	Spleen	Liver
1 (negative control mouse IgG2a, n=8)	4.08, 1.5-4.50	2.42, 2.0-2.67
2 (7 mg sheep polyclonal IgG anti-human SAP antibody, n=5)	1.17, 1.0-1.5	1.0, 0.67-1.17
3 (1 mg monoclonal mouse IgG2a anti-human SAP antibody, SAP-K, n=10)	3.5, 2.83-4.5	1.83, 1.0-2.83
4 (5 mg monoclonal mouse IgG2a anti-human SAP antibody, SAP-K, n=10)	1.25, 1.0-2.0	1.0, 1.0-1.33

Kruskal-Wallis test: spleen, $P < 0.001$; liver $P = 0.001$
Mann-Whitney tests*: 1 vs 2, spleen, $P = 0.002$; liver, $P = 0.002$; 1 vs 3, spleen, $P = 0.173$; liver, $P = 0.083$; 1 vs 4, spleen, $P < 0.001$; liver, $P < 0.001$; 2 vs 3, spleen, $P = 0.001$; liver, $P = 0.019$; 2 vs 4, spleen, $P = 0.513$; liver, $P = 0.768$; 3 vs 4, spleen, $P < 0.001$; liver, $P = 0.004$. *Due to the multiple comparisons, a P value of 0.01 or less is required for significance.

Discussion

[0288] These results demonstrate the efficacy in clearing amyloid deposits *in vivo* of a monoclonal anti-human SAP antibody, of the complement activating mouse IgG2a isotype, which specifically recognizes a conformational epitope. Thus monoclonal anti-human SAP antibodies for use according to the present invention can be directed at either predominantly sequence epitopes, such as antibody SAP-E, or at entirely conformational epitopes, such as SAP-K.

Example 16: Comparison of efficacy of SAP-E and SAP-K in clearing systemic AA amyloid deposits in mice, and estimation of plasma anti-SAP antibody concentrations.

Induction of AA amyloidosis and treatment

[0289] AA amyloidosis was induced and confirmed in wild-type C57BL/6 mice as detailed in Example 10 above. After loading the amyloid deposits with human SAP also detailed in Example 10, groups of mice were treated with 3 mg and 1 mg per mouse of the two different antibodies. A control group, in which amyloid was also induced, received just PBS instead of antibody and two further groups were given the known effective dose of 5 mg/mouse of each antibody. All mice were bled for assay of circulating anti-SAP antibody at days 1, 5 and 15 after dosing with antibody, and all were killed on day 21 for estimation of amyloid load by Congo red staining. All sera were assayed for anti-SAP activity using a robust immunoradiometric assay standardised with purified SAP-E and SAP-K respectively, spiked at known concentrations into normal mouse serum.

Results

[0290] Amyloid load was scored by four independent expert observers all blinded to the identity of each tissue examined. The scores of all observers were, as usual highly concordant and for statistical analysis, the total scores of all observers for both spleen and liver for each mouse were summed. Both antibodies were efficacious, as previously demonstrated, and there was a clear dose dependent effect but SAP-E was apparently more potent than SAP-K at the lower doses.

Table 14. Comparison of potency between SAP-E and SAP-K in clearing visceral AA amyloid deposits

Group (treatment, no. of mice)	Spleen plus liver amyloid score median, range
C (negative control, PBS only)	6.81, 4.25-8.0
K5 (SAP-K 5 mg, n=5)	2.25, 2.25-2.5
K3 (SAP-K 3 mg, n=10)	2.81, 2.0-4.25
K1 (SAP-K 1 mg, n=10)	5.63, 4.0-6.5
E5 (SAP-E 5 mg, n=5)	2.0, 1.5-2.38
E3 (SAP-E 3 mg, n=10)	2.5, 2.0-5.0
E1 (SAP-E 1 mg, n=10)	3.38, 2.5-5.63

Kruskal-Wallis test: $P < 0.001$
Mann-Whitney tests*: K5 vs E5, $P = 0.095$; K3 vs E3, $P = 0.684$; K1 vs E1, $P = 0.001$; K5 vs K3, $P = 0.594$; K5 vs K1, $P = 0.001$; K3 vs K1, $P < 0.001$; E5 vs E3, $P = 0.008$; E5 vs E1, $P = 0.001$; E3 vs E1, $P = 0.004$; K5 vs C, $P = 0.001$; E5 vs C, $P = 0.001$; K3 vs C, $P < 0.001$; E3 vs C, $P < 0.001$; K1 vs C, $P = 0.043$; E1 vs C, $P < 0.001$. *Due to the multiple comparisons, a P value of 0.01 or less is required for significance.

[0291] The concentrations of circulating anti-SAP antibody activity were strongly and consistently dose dependent after the single dose administered to all animals, apart from a single outlying individual in each of the lower dose groups. After the 1 mg per mouse dose, nothing above background was generally detectable even at day 1 in most mice. In contrast, after the 5 mg dose abundant antibody was still present at 15 days, and after 3 mg most mice had circulating antibody at day 5 but few after 15 days (Table 15). There was no significant difference between the patterns for SAP-E and SAP-K.

Table 15. Serum concentration of anti-SAP antibody after single intraperitoneal doses.

Group (dose of anti-SAP antibody)	anti-SAP concentration after dosing median, range ($\mu\text{g/ml}$)*		
	1 day	5 days	15 days
K5 (SAP-K 5 mg)	950, 840-1200	400, 300-480	45, 25-90
E5 (SAP-E 5 mg)	1000, 800-1500	600, 360-700	80, 15-113
K3 (SAP-K 3 mg)	240, 50-600	40, 8-280	8, 6-30
E3 (SAP-E 3 mg)	275, 4-480	48, 0-240	4, 2-68
K1 (SAP-K 1mg)	7, 7-90	6, 5-38	4, 2-9
E1 (SAP-E 1mg)	7, 6-280	7, 6-120	5, 3-12
C (PBS only)	5, 5-7	5, 5-13	5, 5-16

*Apparent anti-SAP antibody concentrations below 17 $\mu\text{g/ml}$ are background for the assay and represent no genuine activity.

Discussion

[0292] In direct head to head comparison there was consistent evidence that SAP-E was slightly but significantly more potent than SAP-K. After administration of 1 mg per mouse no circulating anti-SAP antibody activity was detectable one day later, having evidently all localised to human SAP within the amyloid deposits. After the 3 mg dose abundant anti-SAP was present in the circulation at day 1 and was still present at day 5. After 5 mg per mouse there was still a significant concentration of anti-SAP in the blood after 15 days. These observations suggest that repeated small doses of anti-SAP antibody may be sufficient to trigger amyloid clearance.

Example 17: Comparison of efficacy of low dose SAP-E and SAP-K in clearing systemic AA amyloid deposits in mice.

Induction of AA amyloidosis and treatment

[0293] AA amyloidosis was induced and confirmed in wild-type C57BL/6 mice as detailed in Example 10 above. After loading the amyloid deposits with human SAP as also detailed in Example 10, groups of mice (n=10 each) were treated with single doses of either 0.5 mg and 1 mg per mouse of the two different antibodies, or 6 repeated doses of 0.15 mg, given at 3 or 4 day intervals. A control group (n=9), in which amyloid was also induced, received just PBS instead of antibody and two further groups (n=3 each) were given the known effective dose of 5 mg/mouse of each antibody. All were killed on day 29 for estimation of amyloid load by Congo red staining.

Results

[0294] The low doses, including the repeated very low dose, showed significant efficacy in reducing amyloid load, especially in the liver. SAP-E was again apparently more potent than SAP-K.

Table 16. Comparison of potency between low doses of SAP-E and SAP-K in clearing visceral AA amyloid deposits

Group	Amyloid score, (median, range)	
	Spleen	Liver
C, negative control PBS only	4.5, 4.0-4.75	3.25, 2.0-4.0
E1, SAP-E 1 mg	1.25, 1.0-4.25	1.0, 0.5-1.25
E0.5, SAP-E 0.5 mg	4.75, 1.0-5.0	1.0, 0.5-3.5
Erep, SAP-E 6x0.15 mg	3.5, 2.0-4.5	0.5, 0.0-3.25
K1, SAP-K 1 mg	4.13, 1.0-5.0	1.0, 0.0-4.0
K0.5, SAP-K 0.5 mg	4.25, 1.75-4.5	1.13, 0.0-2.75
Krep, SAP-K 6x0.15 mg	4.38, 1.5-4.75	1.0, 0.0-2.25

Kruskal-Wallis test: spleen, $P < 0.001$; liver, $P = 0.001$

Mann-Whitney tests*: E1 vs C: spleen, $P < 0.001$; liver $P < 0.001$; E0.5 vs C: spleen, $P = 0.604$; liver $P = 0.004$; Erep vs C: spleen, $P = 0.002$; liver, $P < 0.001$; K1 vs C: spleen, $P = 0.065$; liver, $P = 0.001$; K0.5 vs C: spleen, $P = 0.022$; liver, $P = 0.001$; Krep vs C: spleen, $P = 0.079$; liver, $P < 0.001$; E1 vs E0.5: spleen, $P = 0.005$; liver $P = 0.143$; E1 vs Erep: spleen, $P = 0.043$; liver, $P = 0.280$; E0.5 vs Erep: spleen, $P = 0.019$; liver, $P = 0.043$; K1 vs K0.5: no significant differences; K1 vs Krep: no significant differences; K0.5 vs Krep: no significant differences; E1 vs K1: spleen, $P = 0.015$; liver, $P = 0.353$; E0.5 vs K0.5: no significant differences; Erep vs Krep: no significant differences. *Due to the multiple comparisons, a P value of 0.01 or less is required for significance.

Discussion

[0295] The significantly greater potency of SAP-E than SAP-K appears to be reproducible. The efficacy of even very low doses when administered repeatedly and the suggestion of greater effects on liver than spleen amyloid deposits are of interest and potential clinical significance.

Example 18: Activation of complement by humanised monoclonal anti-human SAP antibodies *in vitro*.

[0296] Complement activation is essential for efficacy of amyloid clearing by anti-human SAP antibodies according to the present invention. The capacity of the humanised monoclonal antibodies, SAP-E H1L1 and SAP-K H3L0, to activate C3 in human and mouse serum was compared *in vitro* by adding different amounts of the isolated pure antibodies to either whole human serum containing a SAP concentration of 30 mg/l, or to whole mouse serum which had been spiked with isolated pure human SAP to this same concentration. In both cases the serum was fresh and complement sufficient and experimental conditions were optimal for complement activation with complement fixation test buffer (CFT) as the diluent.

[0297] The following mixtures were made (Table 17):

Tube no.	Serum	Monoclonal anti-SAP antibody	Final concentrations (µg/ml)	
			Anti-SAP	Human SAP
M1	Mouse + human SAP	SAP-E H1L1	15	30
M2	Mouse + human SAP	SAP-E H1L1	30	30
M3	Mouse + human SAP	SAP-E H1L1	60	30
M4	Mouse + human SAP	SAP-E H1L1	120	30
M5	Mouse + human SAP	SAP-K H3L0	15	30
M6	Mouse + human SAP	SAP-K H3L0	30	30
M7	Mouse + human SAP	SAP-K H3L0	60	30
M8	Mouse + human SAP	SAP-K H3L0	120	30
M9	Mouse + human SAP	None	0	30
H1	Human	SAP-E H1L1	15	30
H2	Human	SAP-E H1L1	30	30
H3	Human	SAP-E H1L1	60	30
H4	Human	SAP-E H1L1	120	30
H5	Human	SAP-K H3L0	15	30
H6	Human	SAP-K H3L0	30	30
H7	Human	SAP-K H3L0	60	30
H8	Human	SAP-K H3L0	120	30
H9	Human	None	0	30

[0298] All tubes were incubated at 37°C for 2 hours to enable complement activation to proceed. Since slow spontaneous activation always occurs in serum, two additional controls were provided, replicates of M9 and H9, designated M10 and H10, which were not incubated but were frozen at -80°C immediately after mixing and then thawed just before assaying for C3 cleavage. Comparison between M/H9 and M/H10 enables distinction between spontaneous C3 cleavage and any additional activation produced by the anti-SAP antibody, as well as any effect of addition of human SAP alone to mouse serum.

[0299] C3 cleavage in human serum was assayed by two dimensional electroimmunophoresis using monospecific antibody against human C3. This method is of low sensitivity for mouse C3 cleavage because the different electrophoretic mobilities of mouse C3 are more difficult to distinguish reliable than is the case with human C3. Mouse C3 cleavage was therefore assayed by agarose gel electrophoresis followed by immunoblotting with monospecific antimouse C3 antibody.

Results

[0300] Both humanised antibodies efficiently activated human complement, evidenced by major dose dependent cleavage of C3, producing reduction in the size of the slower mobility native C3 immunoprecipitation peak and increase in the size of the faster cleaved C3c peak (Figure 10).

[0301] Figure 10 shows C3 activation by humanised monoclonal anti-human SAP antibodies in whole human serum.

[0302] In an assay including the control for baseline C3 cleavage in sample H10, it is clear that even the lowest dose of both anti-SAP antibodies produces more C3 cleavage than seen in the no antibody, spontaneous cleavage, control (Figure 11).

[0303] Figure 11 shows C3 activation by low dose humanised monoclonal anti-human SAP antibodies in whole human serum.

[0304] Very similar results were obtained for cleavage of mouse C3 in whole mouse serum supplemented with human SAP. Both antibodies showed dose dependent cleavage of native mouse C3 leading to decreased intensity of the slow mobility native C3 band and increased intensity of the faster mobility activated form. Also even the lowest dose of each antibody produced more C3 cleavage than was seen in the no antibody, spontaneous activation, control (Figure 12).

[0305] Figure 12 shows C3 activation by humanised monoclonal anti-human SAP antibodies in whole mouse serum supplemented with pure human SAP.

Discussion

[0306] Both humanised monoclonal anti-human SAP antibodies efficiently activate complement in the presence of human SAP and are thus suitable candidates for use in treatment of systemic amyloidosis, and any other disease caused by extracellular amyloid deposits in the tissues, according to the present invention.

SEQUENCE CONCORDANCE

SEQ ID NO	Sequence description
1	SAP-E CDRH1 amino acid sequence
2	SAP-E CDRH2 amino acid sequence
3	SAP-E CDRH3 amino acid sequence
4	SAP-E CDRL1 amino acid sequence
5	SAP-E CDRL2 amino acid sequence
6	SAP-E CDRL3 amino acid sequence
7	SAP-E V _H amino acid sequence
8	SAP-E V _H DNA sequence
9	SAP-E V _L amino acid sequence
10	SAP-E V _L DNA sequence
11	SAP-K CDRH1 amino acid sequence
12	SAP-K CDRH2 amino acid sequence
13	SAP-K CDRH3 amino acid sequence
14	SAP-K CDRL1 amino acid sequence
15	SAP-K CDRL2 amino acid sequence
16	SAP-K CDRL3 amino acid sequence
17	SAP-K V _H amino acid sequence
18	SAP-K V _H DNA sequence
19	SAP-K V _L amino acid sequence
20	SAP-K V _L DNA sequence
21	SAP-E V _H chimera amino acid sequence

SEQ ID NO	Sequence description
22	SAP-E V _L chimera amino acid sequence
23	SAP-K V _H chimera amino acid sequence
24	SAP-K V _L chimera amino acid sequence
25	IGHV1-69 human variable heavy chain germline acceptor amino acid sequence
26	JH1 minigene
27	SAP-E humanised V _H variant H0 amino acid sequence
28	SAP-E humanised V _H variant H1 amino acid sequence
29	SAP-E humanised V _H variant H2 amino acid sequence
30	SAP-E humanised V _H variant H3 amino acid sequence
31	SAP-E humanised V _H variant H4 amino acid sequence
32	IGKV1-39 human variable light chain germline acceptor amino acid sequence
33	JK2 minigene
34	SAP-E humanised V _L variant L0 amino acid sequence
35	SAP-E humanised V _L variant L1 amino acid sequence
36	SAP-E humanised V _L variant L2 amino acid sequence
37	SAP-K humanised V _H variant H0 amino acid sequence
38	SAP-K humanised V _H variant H1 amino acid sequence
39	SAP-K humanised V _H variant H2 amino acid sequence
40	SAP-K humanised V _H variant H3 amino acid sequence
41	SAP-K humanised V _L variant L0 amino acid sequence
42	SAP-K humanised V _L variant L1 amino acid sequence
43	Homo sapiens SAP amino acid sequence
44	Mus musculus SAP amino acid sequence
45	SAP-E V _H chimera nucleotide sequence
46	SAP-E V _L chimera nucleotide sequence
47	SAP-K V _H chimera nucleotide sequence
48	SAP-K V _L chimera nucleotide sequence
49	IGHV1-69 human variable heavy chain germline acceptor nucleotide sequence
50	IGHV1-39 human variable heavy chain germline acceptor nucleotide sequence
51	SAP-E humanised heavy chain V region variant H0 nucleotide sequence non-codon optimised
52	SAP-E humanised light chain V region variant L0 nucleotide sequence non-codon optimised
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54	SAP-E humanised heavy chain V region variant H1 nucleotide sequence (codon optimised)
55	SAP-E humanised heavy chain V region variant H2 nucleotide sequence (codon optimised)
56	SAP-E humanised heavy chain V region variant H3 nucleotide sequence (codon optimised)
57	SAP-E humanised heavy chain V region variant H4 nucleotide sequence (codon optimised)
58	SAP-E humanised light chain V region variant L0 nucleotide sequence (codon optimised)
59	SAP-E humanised light chain V region variant L1 nucleotide sequence (codon optimised)
60	SAP-E humanised light chain V region variant L2 nucleotide sequence (codon optimised)
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62	SAP-E humanised heavy chain H1 full mature amino acid sequence
63	SAP-E humanised light chain L1 full mature nucleotide sequence (codon optimised)

SEQ ID NO	Sequence description
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68	SAP-K humanised heavy chain V region variant H1 nucleotide sequence (codon optimised)
69	SAP-K humanised heavy chain V region variant H2 nucleotide sequence (codon optimised)
70	SAP-K humanised heavy chain V region variant H3 nucleotide sequence (codon optimised)
71	SAP-K humanised light chain V region variant L0 nucleotide sequence (codon optimised)
72	SAP-K humanised light chain V region variant L1 nucleotide sequence (codon optimised)
73	SAP-K humanised light chain V region variant L0 91A nucleotide sequence (codon optimised)
74	SAP-K humanised light chain V region variant L0 91A amino acid sequence
75	SAP-K humanised H3 heavy chain nucleotide sequence (codon optimised)
76	SAP-K humanised H3 heavy chain amino acid sequence
77	SAP-K humanised L0 light chain nucleotide sequence (codon optimised)
78	SAP-K humanised L0 light chain amino acid sequence
79	Signal sequence for immunoglobulin chains

SEQUENCE LISTING

[0307]

<110> Glaxo Group Limited
 Tejinder Kaur BHINDER
 Susannah Karen FORD
 Volker GERMASCHEWSKI
 Alan Peter LEWIS
 Mark PEPYS

<120> Anti-SAP mab

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<151> 2010-03-03

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Ser Val Lys Met Ser Cys Lys Ala Ser Gly Phe Thr Phe Ala Thr Tyr
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Asn Met His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile
 35          40          45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
 50          55          60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Asn Thr Ala Tyr
 65          70          75          80
Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85          90          95
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Leu Ala Trp Tyr Gln Gln Lys Gln Gly Arg Ser Pro Gln Leu Leu Val
 35          40          45
His Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Val Ser Gly
 50          55          60
Ser Gly Ser Gly Thr His Phe Ser Leu Lys Ile Asn Gly Leu Gln Pro
 65          70          75          80
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ggaagatccc ctacagtcct ggtocataat gcaaaaacct tagcagaagg tgtgccatca 180
agggtcagtg gcagtggatc aggcacacac ttttctctga agatcaacgg cctgcagcct 240
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1				5				

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Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35          40          45
Gly Met Ile His Pro Asn Ser Val Asn Thr Asn Tyr Asn Glu Lys Phe
 50          55          60
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65          70          75          80
Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85          90          95
Ala Arg Arg Asn Asp Tyr Tyr Trp Tyr Phe Asp Val Trp Gly Thr Gly
100          105          110
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cctggacaag gccttgagtg gattggaatg attcatccta atagtgttaa tactaactac 180
aatgagaagt tcaagagtaa ggccacactg actgtagaca aatcctccag cacagcctac 240
atgcaactca acagcctgac atctgaggac tctgcggtct attactgtgc aagacggaat 300
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Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Asn Ser Asn
 20          25          30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile

          35          40          45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
 50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
 65          70          75          80
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Cys Asn Asn Tyr Pro Phe
 85          90          95
Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys
100          105

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gggcaatctc ctaaagcact gatttactcg gcttcctacc ggtacagtgg agtccctgat 180
cgcttcacag gcagtgatc tgggacagat ttactctca ccatcaccaa tgtgcagtct 240
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<212> PRT

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20     25     30
Asn Met His Trp Ile Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile
35     40     45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
50     55     60
Lys Gly Lys Ala Thr Leu Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65     70     75     80
Met Gln Ile Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85     90     95
Arg Asp Phe Asp Tyr Asp Gly Gly Tyr Tyr Phe Asp Ser Trp Gly Gln
100    105    110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115    120    125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130    135    140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145    150    155    160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165    170    175
Leu Gln Ser Ser Gly Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180    185    190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195    200    205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
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His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser

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260      265      270
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275      280      285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Tyr Val Val Ser Val
290      295      300
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305      310      315      320
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325      330      335
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340      345      350
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355      360      365
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370      375      380
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385      390      395      400
Gly Ser Phe Phe Lys Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
405      410      415
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<210> 22

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<212> PRT

<213> Artificial Sequence

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20      25      30
Leu Ala Trp Tyr Gln Gln Lys Gln Gly Arg Ser Pro Gln Leu Leu Val
35      40      45
His Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Val Ser Gly
50      55      60
Ser Gly Ser Gly Thr His Phe Ser Leu Lys Ile Asn Gly Leu Gln Pro
65      70      75      80
Glu Asp Phe Gly Asn Tyr Tyr Cys Gln His His Tyr Gly Ala Pro Leu
85      90      95
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg Thr Val Ala Ala
100      105      110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115      120      125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130      135      140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145      150      155      160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165      170      175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180      185      190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
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Phe Asn Arg Gly Glu Cys

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210

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35
Gly Met Ile His Pro Asn Ser Val Asn Thr Asn Tyr Asn Glu Lys Phe
50
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65      70      75      80
Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85      90      95
Ala Arg Arg Asn Asp Tyr Tyr Trp Tyr Phe Asp Val Trp Gly Thr Gly
100      105      110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115      120      125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130      135      140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145      150      155      160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165      170      175
Gln Ser Ser Gly Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180      185      190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195      200      205
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210      215      220
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225      230      235      240
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245      250      255
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260      265      270
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275      280      285
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Tyr Val Val Ser Val Leu
290      295      300
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305      310      315      320
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325      330      335
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340      345      350
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355      360      365
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370      375      380
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385      390      395      400
Ser Phe Phe Lys Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
405      410      415
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
420      425      430
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435      440

```

<210> 24

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K VL chimera amino acid sequence

<400> 24

```

Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1      5      10      15
Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Asn Ser Asn
20     25     30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
35     40     45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Asp Arg Phe Thr Gly
50     55     60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Asn Val Gln Ser
65     70     75     80
Glu Asp Leu Ala Glu Tyr Phe Cys Gln Gln Cys Asn Asn Tyr Pro Phe
85     90     95
Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100    105    110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115    120    125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130    135    140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145    150    155    160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165    170    175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180    185    190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195    200    205
Phe Asn Arg Gly Glu Cys
210

```

<210> 25

<211> 98

<212> PRT

<213> Homo sapiens sequence

<220>

<223> IGHV1-69 human variable heavy chain germline

acceptor amino acid sequence

<400> 25

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20     25     30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35     40     45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50     55     60
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65     70     75     80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

```

Ala Arg

85

90

95

<210> 26

<211> 17

<212> PRT

<213> Homo sapiens sequence

<220>

<223> JH1 minigene

<400> 26

```

Ala Glu Tyr Phe Gln His Trp Gly Gln Gly Thr Leu Val Thr Val Ser
1      5      10      15
Ser

```

<210> 27

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VH variant H0 amino acid sequence

<400> 27

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Thr Tyr
 20      25      30
Asn Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35      40      45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
 50      55      60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Thr Ala Tyr
 65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85      90      95
Arg Asp Phe Asp Tyr Asp Gly Gly Tyr Tyr Phe Asp Ser Trp Gly Gln
100      105      110
Gly Thr Leu Val Thr Val Ser Ser
115      120

```

<210> 28

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VH variant H1 amino acid sequence

<400> 28

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Thr Phe Ala Thr Tyr
 20      25      30
Asn Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35      40      45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
 50      55      60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr

```

```

65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85      90      95
Arg Asp Phe Asp Tyr Asp Gly Gly Tyr Tyr Phe Asp Ser Trp Gly Gln
100      105      110
Gly Thr Leu Val Thr Val Ser Ser
115      120

```

<210> 29

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VH variant H2 amino acid sequence

<400> 29

```

Gln Ala Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Thr Phe Ala Thr Tyr
 20      25      30
Asn Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35      40      45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
 50      55      60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85      90      95
Arg Asp Phe Asp Tyr Asp Gly Gly Tyr Tyr Phe Asp Ser Trp Gly Gln
100      105      110
Gly Thr Leu Val Thr Val Ser Ser
115      120

```

<210> 30

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VH variant H3 amino acid sequence

<400> 30

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Thr Phe Ala Thr Tyr
 20          25          30
Asn Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35          40          45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
 50          55          60
Lys Gly Arg Ala Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85          90          95
Arg Asp Phe Asp Tyr Asp Gly Gly Tyr Tyr Phe Asp Ser Trp Gly Gln
100          105          110
Gly Thr Leu Val Thr Val Ser Ser
115          120

```

<210> 31

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VH variant H4 amino acid sequence

<400> 31

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Thr Phe Ala Thr Tyr
 20          25          30
Asn Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35          40          45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
 50          55          60
Lys Gly Arg Ala Thr Leu Thr Ala Asp Thr Ser Thr Ser Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
 85          90          95
Arg Asp Phe Asp Tyr Asp Gly Gly Tyr Tyr Phe Asp Ser Trp Gly Gln
100          105          110
Gly Thr Leu Val Thr Val Ser Ser
115          120

```

<210> 32

<211> 95

<212> PRT

<213> Homo sapiens sequence

<220>

<223> IGKV1-39 human variable light chain germline
acceptor amino acid sequence

<400> 32

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1          5          10          15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
 20          25          30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35          40          45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65          70          75          80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro
 85          90          95

```

<210> 33

<211> 12

<212> PRT

<213> Homo sapiens sequence

<220>

<223> JK2 minigene

<400> 33

```

Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 1          5          10

```

<210> 34

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VL variant L0 amino acid sequence

<400> 34

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10          15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr
 20          25          30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35          40          45
Tyr Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
 50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65          70          75          80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Gly Ala Pro Leu
 85          90          95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100          105

```

<210> 35

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VL variant L1 amino acid sequence

<400> 35

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10          15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr
 20          25          30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35          40          45
His Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
 50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65          70          75          80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Gly Ala Pro Leu
 85          90          95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100          105

```

<210> 36

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised VL variant L2 amino acid sequence

<400> 36

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10          15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr
 20          25          30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Val
 35          40          45
His Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
 50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65          70          75          80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Gly Ala Pro Leu
 85          90          95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100          105

```

<210> 37

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised VH variant H0 amino acid sequence

<400> 37

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
 20          25          30
Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35          40          45
Gly Met Ile His Pro Asn Ser Val Asn Thr Asn Tyr Asn Glu Lys Phe
 50          55          60
Lys Ser Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85          90          95
Ala Arg Arg Asn Asp Tyr Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly
100          105          110
Thr Leu Val Thr Val Ser Ser
115

```

<210> 38

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised VH variant H1 amino acid sequence

<400> 38

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20          25          30
Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35          40          45
Gly Met Ile His Pro Asn Ser Val Asn Thr Asn Tyr Asn Glu Lys Phe
 50          55          60
Lys Ser Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85          90          95
Ala Arg Arg Asn Asp Tyr Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly
100          105          110
Thr Leu Val Thr Val Ser Ser
115

```

<210> 39

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised VH variant H2 amino acid sequence

<400> 39

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1          5          10          15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20          25          30
Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35          40          45
Gly Met Ile His Pro Asn Ser Val Asn Thr Asn Tyr Asn Glu Lys Phe
 50          55          60
Lys Ser Arg Ala Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85          90          95
Ala Arg Arg Asn Asp Tyr Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly
100          105          110
Thr Leu Val Thr Val Ser Ser
115

```

<210> 40

<211> 119

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised VH variant H3 amino acid sequence

<400> 40

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
 1          5          10
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20          25          30
Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35          40          45
Gly Met Ile His Pro Asn Ser Val Asn Thr Asn Tyr Asn Glu Lys Phe
 50          55          60
Lys Ser Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85          90          95
Ala Arg Arg Asn Asp Tyr Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly
100          105          110
Thr Leu Val Thr Val Ser Ser
115

```

<210> 41

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised VL variant L0 amino acid sequence

<400> 41

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1          5          10          15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asn Ser Asn
 20          25          30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35          40          45

Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
 50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65          70          75          80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Cys Asn Asn Tyr Pro Phe
 85          90          95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100          105

```

<210> 42

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised VL variant L1 amino acid sequence

<400> 42

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1          5          10          15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asn Ser Asn
 20          25          30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
 35          40          45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
 50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65          70          75          80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Cys Asn Asn Tyr Pro Phe
 85          90          95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100          105

```

<210> 43

<211> 204

<212> PRT

<213> Homo sapiens sequence

<220>

<223> Homo sapiens SAP amino acid sequence

<400> 43

```

His Thr Asp Leu Ser Gly Lys Val Phe Val Phe Pro Arg Glu Ser Val
 1      5      10      15
Thr Asp His Val Asn Leu Ile Thr Pro Leu Glu Lys Pro Leu Gln Asn
 20     25     30
Phe Thr Leu Cys Phe Arg Ala Tyr Ser Asp Leu Ser Arg Ala Tyr Ser
 35     40     45
Leu Phe Ser Tyr Asn Thr Gln Gly Arg Asp Asn Glu Leu Leu Val Tyr
 50     55     60
Lys Glu Arg Val Gly Glu Tyr Ser Leu Tyr Ile Gly Arg His Lys Val
 65     70     75     80
Thr Ser Lys Val Ile Glu Lys Phe Pro Ala Pro Val His Ile Cys Val
 85     90     95
Ser Trp Glu Ser Ser Ser Gly Ile Ala Glu Phe Trp Ile Asn Gly Thr
100    105    110
Pro Leu Val Lys Lys Gly Leu Arg Gln Gly Tyr Phe Val Glu Ala Gln
115    120    125
Pro Lys Ile Val Leu Gly Gln Glu Gln Asp Ser Tyr Gly Gly Lys Phe
130    135    140
Asp Arg Ser Gln Ser Phe Val Gly Glu Ile Gly Asp Leu Tyr Met Trp
145    150    155    160
Asp Ser Val Leu Pro Pro Glu Asn Ile Leu Ser Ala Tyr Gln Gly Thr

          165          170          175
Pro Leu Pro Ala Asn Ile Leu Asp Trp Gln Ala Leu Asn Tyr Glu Ile
180    185    190
Arg Gly Tyr Val Ile Ile Lys Pro Leu Val Trp Val
195    200

```

<210> 44

<211> 203

<212> PRT

<213> Mus musculus sequence

<220>

<223> Mus musculus SAP amino acid sequence

<400> 44

```

Gln Thr Asp Leu Lys Arg Lys Val Phe Val Phe Pro Arg Glu Ser Glu
 1      5      10      15
Thr Asp His Val Lys Leu Ile Pro His Leu Glu Lys Pro Leu Gln Asn
 20     25     30
Phe Thr Leu Cys Phe Arg Thr Tyr Ser Asp Leu Ser Arg Ser Gln Ser
 35     40     45
Leu Phe Ser Tyr Ser Val Lys Gly Arg Asp Asn Glu Leu Leu Ile Tyr
 50     55     60
Lys Glu Lys Val Gly Glu Tyr Ser Leu Tyr Ile Gly Gln Ser Lys Val
 65     70     75     80
Thr Val Arg Gly Met Glu Glu Tyr Leu Ser Pro Val His Leu Cys Thr
 85     90     95
Thr Trp Glu Ser Ser Ser Gly Ile Val Glu Phe Trp Val Asn Gly Lys
100    105    110
Pro Trp Val Lys Lys Ser Leu Gln Arg Glu Tyr Thr Val Lys Ala Pro
115    120    125
Pro Ser Ile Val Leu Gly Gln Glu Gln Asp Asn Tyr Gly Gly Gly Phe
130    135    140
Gln Arg Ser Gln Ser Phe Val Gly Glu Phe Ser Asp Leu Tyr Met Trp
145    150    155    160
Asp Tyr Val Leu Thr Pro Gln Asp Ile Leu Phe Val Tyr Arg Asp Ser
165    170    175
Pro Val Asn Pro Asn Ile Leu Asn Trp Gln Ala Leu Asn Tyr Glu Ile
180    185    190
Asn Gly Tyr Val Val Ile Arg Pro Arg Val Trp
195    200

```

<210> 45

<211> 1356

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E VH chimera nucleotide sequence

<400> 45

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ccgggacagg gcctggaatg gattgggtat atttatcctg gagatggtaa tgctaactac 180
aatcagcagt tcaaggggcaa ggccacattg actgcagaca catcctccaa cacagcctac 240
atgcagatca gcagcctgac atctgaagac tctgcggtct atttctgtgc aagaggggac 300
tttgattacg acggagggta ctactttgac tcctggggcc agggcacact agtgaccotg 360
tccagcgcca gcaccaaggg ccccgagctg tccccctgg cccccagcag caagagcacc 420
agcggcgcca cagccgccct gggctgcctg gtgaaggact acttccccga accggtgacc 480
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agcagcggcc tgtacagcct gagcagcgtg gtgaccgtgc ccagcagcag cctgggcacc 600
cagacctaca tctgtaacgt gaaccacaag cccagcaaca ccaaggtgga caagaaggtg 660
gagcccaaga gctgtgacaa gaccacacac tgccccccct gccctgcccc cgagctgctg 720

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```

ggaggcccca gcgtgttccct gttccccccc aagcctaagg acaccctgat gatcagcaga 780
acccccgagg tgacctgtgt ggtggtggat gtgagccacg aggaccctga ggtgaagttc 840
aactcgttacg tggacggcgt ggaggtgcac aatgccaaaga ccaagcccag ggaggagcag 900
tacaacagca cctaccgggt ggtgtccgtg ctgaccgtgc tgcaccagga ttggctgaac 960
ggcaaggagt acaagtgtaa ggtgtccaaac aaggccctgc ctgcccttat cgagaaaacc 1020
atcagcaagg ccaagggccca gccacagagag ccccaggtgt acaccctgcc ccctagcaga 1080
gatgagctga ccaagaacca ggtgtccctg acctgccttg tgaagggctt ctacccccagc 1140
gacatcgccg tggagtggga gagcaacggc cagcccagga acaactacaa gaccaccccc 1200
cctgtgctgg acagcgatgg cagcttcttc ctgtacagca agctgaccgt ggacaagagc 1260
agatggcagc agggcaacgt gttcagctgc tccgtgatgc acgaggccct gcacaatcac 1320
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```

<210> 46

<211> 642

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E VL chimera nucleotide sequence

<400> 46

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atcacatgtc gagcaagtga gaatatttac agttatttag catggtatca gcagaaacag 120
ggagatcccc ctcagctcct ggtccataat gcaaaaacct tagcagaagg tgtgccatca 180
aggtgcagtg gcagtggtac aggcacacac tttctctga agatcaacgg cctgcagcct 240
gaagattttg ggaattatta ctgtcaacat cattatgggt ctcgcctcac gttcgggtgt 300
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agcgatgagc agctgaagag cggcacccgc agcgtggtgt gctcgtgtaa caactctac 420
ccccgggagg ccaaggtgca gtggaagggt gacaatgcc tgcagagcgg caacagccag 480
gagagcgtga ccgacagcga cagcaaggac tccacctaca gcctgagcag caccctgacc 540
ctgagcaagg ccgactacga gaagcacaag gtgtacgcct gtgaggtgac ccaccagggc 600
ctgtccagcc ccgtgaccaa gagcttcaac cggggcgagt gc 642

```

<210> 47

<211> 1347

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K VH chimera nucleotide sequence

<400> 47

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cctggacaag gccttgagtg gattggaatg attcatccta atagtgttaa tactaactac 180
aatgagaagt tcaagagtaa ggcacactg actgtagaca aatcctccag cacagcctac 240
atgcaactca acagcctgac atctgaggac tctgcggtct attactgtgc aagacggaat 300
gattactact ggtacttcga tgtctggggc acagggacac tagtgaccgt gtccagcgcc 360
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acagccgccc tgggctgcct ggtgaaggac tacttccccg aaccggtgac cgtgtcctgg 480
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ctgtacagcc tgagcagcgt ggtgaccgtg cccagcagca gcctgggac ccaagcctac 600
atctgtaacg tgaaccacaa gccacgcaac accaaggtgg acaagaaggt ggagcccaag 660
agctgtgaca agaccacac ctgccccccc tgccctgccc ccgagctgct gggaggcccc 720
agcgtgttcc tgttcccccc caagcctaag gacaccctga tgatcagcag aacccccgag 780
gtgacctgtg tgggtgggtgga tgtgagccac gaggaacctg aggtgaagtl caactgggtac 840
gtggacggcg tggagggtgca caatgccaa accaagccca gggaggagca gtacaacagc 900
acctaccggg tgggtgocgt gctgaccgtg ctgcaccagg attggctgaa cggcaaggag 960
tacaagtgtg aggtgtccaa caaggccctg cctgccccta tcgagaaaac catcagcaag 1020
gccaaaggcc agcccagaga gcccccaggtg tacaccctgc cccctagcag agatgagctg 1080
accaagaacc aggtgtccct gacctgcctg gtgaagggtc tctaccccag cgacatcgcc 1140
gtggagtggg agagcaacgg ccagcccag aacaactaca agaccacccc cctgtgctgt 1200
gacagcgtat gcagcttctt cctgtacagc aagctgaccg tggacaagag cagatggcag 1260
caggccaacg tgttcagctg ctccgtgatg cagcaggccc tgcacaatca ctaccccag 1320
aagagcctga gcctgtcccc tggcaag 1347

```

<210> 48

<211> 642

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K VL chimera nucleotide sequence

<400> 48

```

gacattgtga tgaccagtc tcaaaaattc atgtccacat cagtaggaga cagggtcagc 60
gtcacctgca aggccagtc gaattgtgac tctaatgtag cctggtatca acagaaacca 120
gggcaatctc ctaaagcact gatttactcg gcttctacc ggtacagtgg agtccctgat 180
cgcttcacag gcagtggtatc tgggacagat ttcactctca ccatcaccaa tgtgcagtct 240
gaagacttgg cagagtattt ctgtcagcaa tgtaacaact atccattcac gttcggctcg 300
gggacaaagt tggaaataaa acgtacggtg gccgcccca gcgtgttcat cttccccc 360
agcgatgagc agctgaagag cggcaccgcc agcgtggtgt gtctgctgaa caacttctac 420
cccggggagg ccaagggtga gtggaagggt gacaatgcc tgcagagcgg caacagccag 480
gagagcgtga ccgagcagga cagcaaggac tccacctaca gcctgagcag caccctgacc 540
ctgagcaagg ccgactacga gaagcacaag gtgtacgcct gtgaggtgac ccaccagggc 600
ctgtccagcc ccgtgaccaa gagcttcaac cggggcgagt gc 642

```

<210> 49

<211> 294

<212> DNA

<213> Homo sapiens sequence

<220>

<223> IGHV1-69 human variable heavy chain germline

acceptor nucleotide sequence

<400> 49

```

caggtgcagc tgggtcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccctcagc agctatgcta tcagctgggt gcgacaggcc 120
cctggacaag ggccttgagt gatgggagg atcatccta tctttggtac agcaactac 180
gcacagaagt tccagggcag agtcacgatt accgcggaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gaga 294

```

<210> 50

<211> 285

<212> DNA

<213> Homo sapiens sequence

<220>

<223> IGHV1-39 human variable heavy chain germline

acceptor nucleotide sequence

<400> 50

```

gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcaactgac gggcaagtca gagcattagc agctatttaa attggtatca gcagaaacca 120
gggaaagccc ctaagctcct gatctatgct gcacccagtt tgcaaaagtgg ggtcccatca 180
aggttcagtg gcagtggtatc tgggacagat ttcactctca ccatcagcag tctgcaacct 240
gaagattttg caacttacta ctgtcaacag agttacagta cccct 285

```

<210> 51

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain V region variant H0

nucleotide sequence non-codon optimised

<400> 51

caggtgcagc tgggtcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60

```

tcctgcaagg cttctggagg caccctcagc acttacaata tgcactgggt gcgacaggcc 120
cctggacaag ggccttgagt gatgggatat atttatcctg gagatggtaa tgctaactac 180
aatcagcagt tcaagggcag agtcacgatt accgcggaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagaggggac 300
tttgattacg acggagggta ctactttgac tcctggggcc agggcaccct ggtcaccgtc 360
tcctca

```

<210> 52

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised light chain V region variant L0

nucleotide sequence non-codon optimised

<400> 52

```

gacatccaga tgaccacgtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcaactggc gagcaagtga gaatatattac agttatttag catggtatca gcagaaacca 120
gggaaagccc ctaagctcct gatctataat gcaaaaacct tagcagaagg ggtcccatca 180
aggttcagtg gcagtggatc tgggacagat ttcaactctca ccatcagcag tctgcaacct 240
gaagattttg caacttacta ctgtcaacat cattatggtg ctccgctcac gtttggccag 300
gggaccaagc tggagatcaa a                                     321

```

<210> 53

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain V region variant H0
nucleotide sequence (codon optimised)

<400> 53

```

caggtgcagc tgggtgcagag cggcgccgag gtgaagaaac ccggcagcag cgtgaaggtg 60
agctgcaagg ctacgggggg caccttctcc acctacaaca tgcactgggt caggcaggca 120
cccgccaggg gcctggagtg gatgggctat atctaccccg gcgacggcaa cgccaactac 180
aaccagcagt tcaagggcag ggtgacctc accgcccaga agagcaccag caccgcctac 240
atggaactga gcagcctgag gagcgaggat accgcccgtgt actactgcgc caggggcgac 300
ttcgactacg acggcggcta ctacttcgac agctggggac agggcacact agtgaccgtg 360
tccagc                                     366

```

<210> 54

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain V region variant H1
nucleotide sequence (codon optimised)

<400> 54

```

caggtgcagc tgggtgcagag cggcgccgag gtgaagaaac ccggcagcag cgtgaaggtg 60
agctgcaagg ctacgggggt caccttcgcc acctacaaca tgcactgggt caggcaggca 120
cccgccaggg gcctggagtg gatgggctat atctaccccg gcgacggcaa cgccaactac 180
aaccagcagt tcaagggcag ggtgacctc accgcccaga agagcaccag caccgcctac 240
atggaactga gcagcctgag gagcgaggat accgcccgtgt actactgcgc caggggcgac 300
ttcgactacg acggcggcta ctacttcgac agctggggac agggcacact agtgaccgtg 360
tccagc                                     366

```

<210> 55

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain V region variant H2 nucleotide sequence (codon optimised)

<400> 55

```

caggtgcagc tgggtgcagag cggcgccgag gtgaagaaac ccggcagcag cgtgaaggtg 60
agctgcaagg ctacgggggt caccttcgcc acctacaaca tgcactgggt caggcaggca 120
cccgccaggg gcctggagtg gatgggctat atctaccccg gcgacggcaa cgccaactac 180
aaccagcagt tcaagggcag ggtgacctc accgcccaga agagcaccag caccgcctac 240
atggaactga gcagcctgag gagcgaggat accgcccgtgt actactgcgc caggggcgac 300
ttcgactacg acggcggcta ctacttcgac agctggggac agggcacact agtgaccgtg 360
tccagc                                     366

```

<210> 56

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain V region variant H3
nucleotide sequence (codon optimised)

<400> 56

```

caggtgcagc tgggtgcagag cggcgccgag gtgaagaaac ccggcagcag cgtgaagggtg 60
agctgcaagg ctacgggggtt cactttcgcc acctacaaca tgcactgggt caggcaggca 120
ccgggccagg gcctggagtg gatcggtat atctaccccg gcgacggcaa cgccaactac 180
aaccagcagt tcaagggcag ggccaccatc accgcccaga agagcaccag caccgcctac 240
atggaactga gcagcctgag gagcgaggat accgccgtgt actactgcgc cagggggcgac 300
ttcgactacg acggcggcta ctacttcgac agctggggac agggcacact agtgaccgtg 360
tccagc 366

```

<210> 57

<211> 366

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain V region variant H4

nucleotide sequence (codon optimised)

<400> 57

```

caggtgcagc tgggtgcagag cggcgccgag gtgaagaaac ccggcagcag cgtgaagggtg 60
agctgcaagg ctacgggggtt cactttcgcc acctacaaca tgcactgggt caggcaggca 120
ccgggccagg gcctggagtg gatcggtat atctaccccg gcgacggcaa cgccaactac 180
aaccagcagt tcaagggcag ggccaccatc accgcccaga ccagcaccag caccgcctac 240
atggaactga gcagcctgag gagcgaggat accgccgtgt acttctgcgc cagggggcgac 300
ttcgactacg acggcggcta ctacttcgac agctggggac agggcacact agtgaccgtg 360
tccagc 366

```

<210> 58

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised light chain V region variant L0

nucleotide sequence (codon optimised)

<400> 58

```

gacatccaga tgaccagag cccagctca ctgagcgcca gcgtgggcca cagggtgacc 60
attacctgca gggcctccga gaacatctac agctacctgg cctggtagca gcagaagccc 120
ggcaaggccc ccaagctgct gatctacaac gccaaagccc tcgccgaggg cgtccctagc 180
aggttctctg gaagcggcag cggcacccgac ttcaacctga ccatcagcag cctgcagccc 240
gaggacttcg ccacctatta ctgccagcac cactacggcg cccccctgac ctttggccag 300
ggcaccaaac tggagatcaa g 321

```

<210> 59

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised light chain V region variant L1

nucleotide sequence (codon optimised)

<400> 59

```

gacatccaga tgaccagag cccagctca ctgagcgcca gcgtgggcca cagggtgacc 60
attacctgca gggcctccga gaacatctac agctacctgg cctggtagca gcagaagccc 120
ggcaaggccc ccaagctgct gatccacaac gccaaagccc tcgccgaggg cgtccctagc 180
aggttctctg gaagcggcag cggcacccgac ttcaacctga ccatcagcag cctgcagccc 240
gaggacttcg ccacctatta ctgccagcac cactacggcg cccccctgac ctttggccag 300
ggcaccaaac tggagatcaa g 321

```

<210> 60

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised light chain V region variant L2

nucleotide sequence (codon optimised)

<400> 60


```

gacatccaga tgacccagag cccagctca ctgagcgcca gcgtggcgga cagggtgacc 60
attacctgca gggcctccga gaacatctac agctacctgg cctggtacca gcagaagccc 120
ggcaaggccc ccaagctgct ggtgcacaac gccaaagccc tcgcgagggg cgtccctagc 180
aggttctctg gaagcggcag cggcaccgac ttaccctga ccatcagcag cctgcagccc 240
gaggacttcg ccacctatta ctgccagcac cactacggcg ccccccctgac ctttggccag 300
ggcaccaaac tggagatcaa g

```

<210> 61

<211> 1356

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain H1 full mature
nucleotide sequence (codon optimised)

<400> 61

```

caggtgcagc tgggtcagag cggcgccgag gtgaagaaac ccggcagcag cgtgaagggtg 60
agctgcaagg ctagcgggtt cacccttcgcc acctacaaca tgcaactgggt caggcaggca 120
cccgccgagg gcctggagtg gatgggctat atctaccccg gcgacggcaa cgccaactac 180
aaccagcagt tcaaggggcag ggtgaccatc accgccgaca agagcaccag caccgcctac 240
atggaactga gcagcctgag gagcgaggat accgccgtgt actactgcgc caggggcgac 300
ttcgactacg acggcggcta ctacttcgac agctggggac agggcacact agtgaccgtg 360
tccagcgcca gcccaaggg cccagcgtg ttccccctgg cccccagcag caagagcacc 420
agcggcggca cagccgcctt gggctgcctg gtgaaggact acctcccga accggtgacc 480
gtgtcctgga acagcggagc cctgaccagc ggcgtgcaca ccttcccgc cgtgctgcag 540
agcagcggcc tgtacagcct gagcagcgtg gtgaccgtgc ccagcagcag cctgggcacc 600
cagacctaca tctgtaacgt gaaccacaag ccagcaaca ccaagggtga caagaagggtg 660
gagcccaaga gctgtgacaa gaccacaccc tgcctccccc gccctgcccc ctagctgctg 720
ggagggccca gcgtgttcct gttccccccc aagcctaagg acaccctgat gatcagcaga 780
accccgagg tgacctgtgt ggtggtggat gtgagccacg aggaccctga ggtgaagtgc 840
aactggtacg tggcggcgtt gtaggtgcac aatgccaaag ccaagcccg ggaggagcag 900
tacaacagca cctacgggtt ggtgtccgtg ctgaccgtgc tgcaccagga ttggctgaac 960
ggcaaggagt acaagtgtaa ggtgtccaac aaggccctgc ctgcccctat cgagaaaacc 1020
atcagcaagg ccaagggcca gccagagag cccaggtgt acaccctgcc cctagcaga 1080
gatgagctga ccaagaacca ggtgtccctg acctgcctgg tgaagggtt ctacccacagc 1140
gacatcgccg tggagtggga gagcaacggc cagcccgaga acaactaca gaccaccccc 1200

```

```

cctgtgctgg acagcgatgg cagcttcttc ctgtacagca agctgaccgt ggacaagagc 1260
agatggcagc agggcaacgt gttcagctgc tccgtgatgc acgagggccc gcacaatcac 1320
tacaccagaa agagcctgag cctgtccctt ggcaag

```

<210> 62

<211> 444

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised heavy chain H1 full mature amino
acid sequence

<400> 62

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Thr Phe Ala Thr Tyr
20      25      30
Asn Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35      40      45
Gly Tyr Ile Tyr Pro Gly Asp Gly Asn Ala Asn Tyr Asn Gln Gln Phe
50      55      60
Lys Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65      70      75      80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85      90      95
Arg Asp Phe Asp Tyr Asp Gly Gly Tyr Tyr Phe Asp Ser Trp Gly Gln
100      105      110
Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115      120      125
Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
130      135      140
Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145      150      155      160
Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165      170      175
Leu Gln Ser Ser Gly Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
180      185      190
Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
195      200      205
Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr
210      215      220
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225      230      235      240
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
245      250      255
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
260      265      270
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
275      280      285
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Tyr Val Val Ser Val
290      295      300
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
305      310      315      320
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
325      330      335
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
340      345      350
Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
355      360      365
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
370      375      380
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
385      390      395      400
Gly Ser Phe Phe Lys Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
405      410      415
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
420      425      430
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435      440

```

<210> 63

<211> 642

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-E humanised light chain L1 full mature

nucleotide sequence

(codon optimised)

<400> 63

```

gacatccaga tgacccagag ccccagctca ctgagcgcca gcgtgggcga cagggtgacc 60
attacctgca gggcctccga gaacatctac agctacctgg cctggtagca gcagaagccc 120
ggcaaggccc ccaagctgct gatccacaac gccaaagccc tcgccgaggg cgtccctagc 180
aggttctctg gaagcggcag cggcaccgac ttcaacctga ccatcagcag cctgcagccc 240
gaggacttcg ccacctatta ctgccagcac cactacggcg ccccctgac ctttggccag 300
ggcaccaaac tggagatcaa gcgtacggtg gccgcccaca gcgtgttcac ctteccccc 360
agcgatgagc agctgaagag cggcaccgccc agcgtggtgt gtctgctgaa caacttctac 420
ccccgggagg ccaaggtgca gtggaagggt gacaatgccc tgcagagcgg caacagccag 480
gagagcgtga ccgagcagga cagcaaggac tccacctaca ccctgagcag caccctgacc 540
ctgagcaagg ccgactacga gaagcacaag gtgtacgcct gtgaggtgac ccaccagggc 600
ctgtccagcc ccgtgaccaa gagcttcaac cggggcgagt gc 642

```

<210> 64

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-E humanised light chain L1 full mature amino acid sequence

<400> 64

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1      5      10      15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Ser Tyr
20     25     30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35     40     45
His Asn Ala Lys Thr Leu Ala Glu Gly Val Pro Ser Arg Phe Ser Gly
50     55     60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65     70     75     80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln His His Tyr Gly Ala Pro Leu
85     90     95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100    105    110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115    120    125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130    135    140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145    150    155    160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165    170    175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180    185    190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195    200    205
Phe Asn Arg Gly Glu Cys
210

```

<210> 65

<211> 357

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised heavy chain V region variant H0
nucleotide sequence non-codon optimised

<400> 65

```

caggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc agctactgga tgcaactgggt gcgacaggcc 120
cctggacaag ggcttgagtg gatgggaatg attcatccta atagtgttaa tactaactac 180
aatgagaagt tcaagagtag agtcacgatt acgcgggaca aatccacgag cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagacggaat 300
gattactact ggtacttcga tgtctggggc cagggcacc c tggtaaccgt ctctctca 357

```

<210> 66

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised light chain V region variant L0
nucleotide sequence non-codon optimised

<400> 66

```

gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgca aggcagtcga gaatgtgaac tctaattgtag cctggatatca gcagaaacca 120
gggaaagccc ctaagctcct gatctattcg gcttcctacc ggtacagtggt ggtcccatca 180
aggttcagtg gcagtggtac tgggacagat ttcactctca ccatcagcag tctgcaacct 240
gaagattttg caacttacta ctgtcagcaa tgtaacaact atccattcac gtttggccag 300
gggaccaagc tggagatcaa a
321

```

<210> 67

<211> 357

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised heavy chain V region variant H0
nucleotide sequence (codon optimised)

<400> 67

```

caggtgcagc tgggtgcagag cggcgccgaa gtgaagaagc ccggcagcag cgtgaaagtg 60
agctgcaagg ccagcggcga aaccttcacc agctactgga tgcactgggt gaggcaggca 120
cccgccagg gcctggagtg gatgggcatg atccacccca acagcgtgaa caccaactac 180
aacgagaagt tcaagagcag agtgaccatc accgcccaca agagcaccag caccgcctat 240
atggagctga gctctctgag gagcgaggat accgccgtgt actactgcgc caggaggaac 300
gactactact ggtacttcga cgtctggggc cagggcacac tagtgaccgt gtccagc 357

```

<210> 68

<211> 357

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised heavy chain V region variant H1
nucleotide sequence (codon optimised)

<400> 68

```

caggtgcagc tgggtgcagag cggcgccgaa gtgaagaagc ccggcagcag cgtgaaagtg 60
agctgcaagg ccagcggcga aaccttcacc agctactgga tgcactgggt gaggcaggca 120
cccgccagg gcctggagtg gatgggcatg atccacccca acagcgtgaa caccaactac 180
aacgagaagt tcaagagcag agtgaccatc accgcccaca agagcaccag caccgcctat 240
atggagctga gctctctgag gagcgaggat accgccgtgt actactgcgc caggaggaac 300
gactactact ggtacttcga cgtctggggc cagggcacac tagtgaccgt gtccagc 357

```

<210> 69

<211> 357

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised heavy chain V region variant H2
nucleotide sequence (codon optimised)

<400> 69

```

caggtgcagc tgggtgcagag cggcgccgaa gtgaagaagc ccggcagcag cgtgaaagtg 60
agctgcaagg ccagcggcga aaccttcacc agctactgga tgcactgggt gaggcaggca 120
cccgccagg gcctggagtg gatcgccatg atccacccca acagcgtgaa caccaactac 180
aacgagaagt tcaagagcag agccaccatc accgcccaca agagcaccag caccgcctat 240
atggagctga gctctctgag gagcgaggat accgccgtgt actactgcgc caggaggaac 300
gactactact ggtacttcga cgtctggggc cagggcacac tagtgaccgt gtccagc 357

```

<210> 70

<211> 357

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised heavy chain V region variant H3
nucleotide sequence (codon optimised)

<400> 70

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caggtgcagc tgggtgcagag cggcgccgaa gtgaagaagc ccggcagcag cgtgaaagtg 60
agctgcaagg ccagcggcga aaccttcacc agctactgga tgcactgggt gaggcaggca 120
cccgccagg gcctggagtg gatcgccatg atccacccca acagcgtgaa caccaactac 180
aacgagaagt tcaagagcag agccaccatg accgtggaca agagcaccag caccgcctat 240
atggagctga gctctctgag gagcgaggat accgccgtgt actactgcgc caggaggaac 300
gactactact ggtacttcga cgtctggggc cagggcacac tagtgaccgt gtccagc 357

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<210> 71

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised light chain V region variant L0
nucleotide sequence (codon optimised)

<400> 71

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gacatccaga tgaccagag cccctcttca ctgagcgcta gcgtgggcga cagggtgacc 60
atcacctgca aggccagcca gaacgtgaac agcaacgtgg cctggtacca gcagaagccc 120
ggcaaaagccc ccaagtcct gatctacagc gccagctaca gatatagcgg cgtgcctagc 180
aggttttagcg gcagcggaag cgggaccgat ttcacctga ccacagcag cctgcagccc 240
gaggacttcg ccacttacta ctgccagcag tgcaacaact accccttcac cttcggccag 300
ggaccaagc tggagatcaa g 321

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<210> 72

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised light chain V region variant L1
nucleotide sequence (codon optimised)

<400> 72

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gacatccaga tgaccagag cccctcttca ctgagcgcta gcgtgggcga cagggtgacc 60
atcacctgca aggccagcca gaacgtgaac agcaacgtgg cctggtacca gcagaagccc 120
ggcaaaagccc ccaagtcct gatctacagc gccagctaca gatatagcgg cgtgcctagc 180
aggttttagcg gcagcggaag cgggaccgat ttcacctga ccacagcag cctgcagccc 240
gaggacttcg ccacttacta ctgccagcag tgcaacaact accccttcac cttcggccag 300
ggaccaagc tggagatcaa g 321

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<210> 73

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised light chain V region variant L0
91A nucleotide sequence (codon optimised)

<400> 73

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gacatccaga tgaccagag cccctcttca ctgagcgcta gcgtgggcga cagggtgacc 60
atcacctgca aggccagcca gaacgtgaac agcaacgtgg cctggtacca gcagaagccc 120
ggcaaaagccc ccaagtcct gatctacagc gccagctaca gatatagcgg cgtgcctagc 180
aggttttagcg gcagcggaag cgggaccgat ttcacctga ccacagcag cctgcagccc 240
gaggacttcg ccacttacta ctgccagcag tgcaacaact accccttcac cttcggccag 300
ggaccaagc tggagatcaa g 321

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<210> 74

<211> 107

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised light chain V region variant L0
91A amino acid sequence

<400> 74

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1      5      10      15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asn Ser Asn
20     25     30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35     40     45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50     55     60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65     70     75     80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ala Asn Asn Tyr Pro Phe
85     90     95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100    105

```

<210> 75

<211> 1347

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised H3 heavy chain nucleotide sequence
(codon optimised)

<400> 75

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agctgcaagg ccagcggcta caccttcacc agctactgga tgcactgggt gaggcaggca 120
cccggccagg gacctggagt gatcggcatg atccacccca acagcgtgaa caccacttac 180
aacgagaagt tcaagagcag agccaccctg accgtggaca agagcaccag caccgcctat 240
atggagctga gctctctgag gagcgaggat accgccgtgt actactgctg caggaggaac 300
gactactact ggtacttcga cgtctggggc cagggcacac tagtgacctg gtccagcgcc 360
agcaccaagg gcccacagct gttccccctg gccccagca gcaagagcac cagcggcggc 420
adagccgccc tgggtgcct ggtgaaggac tacttccccg aaccggtgac cgtgtcctgg 480
aacagcggag ccctgaccag cggcgtgcac accttccccg ccgtgctgca gagcagcggc 540
ctgtacagcc tgagcagcgt ggtgaccgtg cccagcagca gcctggggcac ccagacctac 600
atctgtaacg tgaaccacaa gcccagcaac accaaggtgg acaagaaggt ggagcccaag 660
agctgtgaca agaccacac ctgccccccc tgccctgccc ccgagctgct gggaggcccc 720
agcgtgttcc tgttcccccc caagcctaag gacacctga tgatcagcag aaccccgag 780
gtgacctgtg tgggtgggga tgtgagccac gaggacctg aggtgaagtt caactggtag 840
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acctaccggg tgggtccgtg gctgaccctg ctgcaccagg attggctgaa cggcaaggag 960
tacaagtgtg aggtgtccaa caaggccctg cctgccccta tcgagaaaac catcagcaag 1020
gccaaaggcc agcccagaga gcccaggtg tacacctgc cccctagcag agatgagctg 1080
accaagaacc aggtgtccct gacctgcctg gtgaagggtc tctacccag cgacatcgcc 1140
gtggagtggg agagcaacgg ccagcccag aacaactaca agaccacccc cctgtgtgctg 1200
gacagcgatg gcagcttctt cctgtacagc aagctgaccg tggacaagag cagatggcag 1260
cagggcaacg tgttcagctg ctccgtgatg cagcaggccc tgcacaatca ctacacccag 1320
aagagcctga gctgtcccc tggcaag 1347

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<210> 76

<211> 443

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised H3 heavy chain amino acid sequence

<400> 76

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1      5      10      15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20     25     30
Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35     40     45
Gly Met Ile His Pro Asn Ser Val Asn Thr Asn Tyr Asn Glu Lys Phe
50     55     60
Lys Ser Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
65     70     75     80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85     90     95
Ala Arg Arg Asn Asp Tyr Tyr Trp Tyr Phe Asp Val Trp Gly Gln Gly
100    105    110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115    120    125
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
130    135    140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145    150    155    160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165    170    175
Gln Ser Ser Gly Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
180    185    190
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
195    200    205
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His
210    215    220
Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val
225    230    235    240
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245    250    255

```

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Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
260    265    270
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275    280    285
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Tyr Val Val Ser Val Leu
290    295    300
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
305    310    315    320
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
325    330    335
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
340    345    350
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
355    360    365
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
370    375    380
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
385    390    395    400
Ser Phe Phe Lys Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
405    410    415
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
420    425    430
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435    440

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<210> 77

<211> 642

<212> DNA

<213> Artificial Sequence

<220>

<223> SAP-K humanised L0 light chain nucleotide sequence

(codon optimised)

<400> 77

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atcacctgca aggccagcca gaacgtgaac agcaacgtgg cctggtagca gcagaagccc 120
ggcaaaagccc ccaagctcct gatctacagc gccagctaca gatatagcgg cgtgcctagc 180
aggtttagcg gcagcgggaag cgggacggat ttcacctga ccatcagcag cctgcagccc 240
gaggacttcg ccacttacta ctgccagcag tgcaacaact accccttcac cttcggccag 300
ggcaccgaagc tggagatcaa gcgtacgggtg gccgcccccga gcgtgttcac ctccccccc 360
agcgatgagc agctgaagag cggcacccgc agcgtggtgt gtctgctgaa caacttctac 420
ccccgggagg ccaaggtgca gtggaagggtg gacaatgccc tgcagagcgg caacagccag 480
gagagcgtga ccgagcagga cagcaaggac tccacctaca gcctgagcag caccctgacc 540
ctgagcaagg ccgactacga gaagcacaag gtgtacgcct gtgaggtgac ccaccagggc 600
ctgtccagcc ccgtgaccaa gagcttcaac cggggcgagt gc 642

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<210> 78

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> SAP-K humanised L0 light chain amino acid sequence

<400> 78

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1      5      10      15
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asn Ser Asn
20     25     30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35     40     45
Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50     55     60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65      70      75      80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Cys Asn Asn Tyr Pro Phe
85      90      95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100     105     110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115     120     125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130     135     140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145     150     155     160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165     170     175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180     185     190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195     200     205
Phe Asn Arg Gly Glu Cys
210

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<210> 79

<211> 19

<212> PRT

<213> Mus musculus

<220>

<223> Signal sequence for immunoglobulin chains

<400> 79

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Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1      5      10      15
Val His Ser

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P A T E N T K R A V

1. Antistof, som specifikt binder til SAP, hvor den tung-kæde variable region er SEQ ID NO: 28, den let-kæde variable region er SEQ ID NO: 35, og hvor antistoffet omfatter et humant konstant domæne af humant IgG1 eller IgG3.
- 5 2. Antistof ifølge krav 1, som omfatter en tung kæde ifølge SEQ ID NO:62 og en let kæde ifølge SEQ ID NO:64.
3. Nukleinsyremolekyle som koder for et antistof som defineret i et hvilket som helst af krav 1-2.
4. Nukleinsyremolekyle ifølge krav 3, omfattende SEQ ID NO:54 og SEQ ID NO:59.
- 10 5. Nukleinsyremolekyle ifølge krav 3, omfattende SEQ ID NO:61 og SEQ ID NO:63.
6. Ekspressionsvektor omfattende et nukleinsyremolekyle ifølge krav 3, 4 eller 5.
7. Rekombinant værtscelle omfattende en ekspressionsvektor ifølge krav 6.
8. Fremgangsmåde til fremstilling af et antistof som defineret i et hvilket som helst af krav 1-2, hvilken fremgangsmåde omfatter trinnet at dyrke en værtscelle ifølge krav 7
- 15 og at indvinde antistoffet.
9. Farmaceutisk sammensætning omfattende et antistof som defineret i et hvilket som helst af krav 1-2 og et farmaceutisk acceptabelt bærestof.
10. Antistof som defineret i et hvilket som helst af krav 1-2 til anvendelse i behandling af eller forebygge en sygdom forbundet med amyloid aflejring, hvor antistoffet er til
- 20 indgivelse med ((2R)-1-[6-[(2R)-2-Carboxypyrrolidin-1-yl]-6-oxohexanoyl]pyrrolidin-2-carboxylsyre; (CPHPC) eller et farmaceutisk acceptabelt salt eller mono- eller diester deraf, hvor indgivelsen af antistoffet og CPHPC er sekventiel, og hvor nævnte CPHPC er til indgivelse først.
11. Antistof til anvendelse ifølge krav 10, hvor antistoffet er til indgivelse når i alt
- 25 væsentlig alle af nævnte SAP, som cirkulerer hos subjektet, er blevet fjernet.
12. Antistof til anvendelse ifølge krav 10 eller 11, hvor sygdommen er valgt fra gruppen bestående af: systemisk amyloidose, lokal amyloidose, Alzheimers sygdom, type-2-diabetes, dialyse-relateret amyloidose, monoklonal immunglobulinkæde (AL)-amyloidose og cerebral amyloid angiopati.

30

DRAWINGS

FIGURES

Figure1

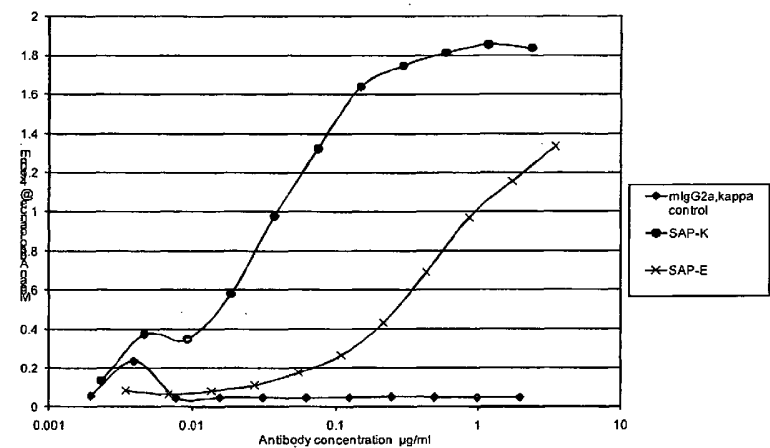


Figure 2

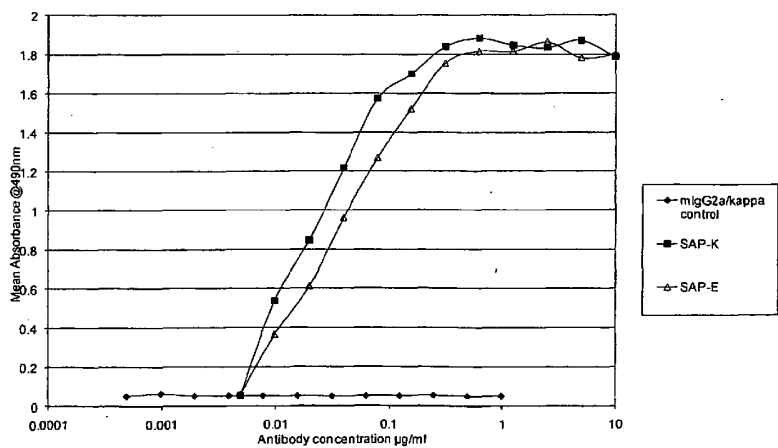


Figure 3

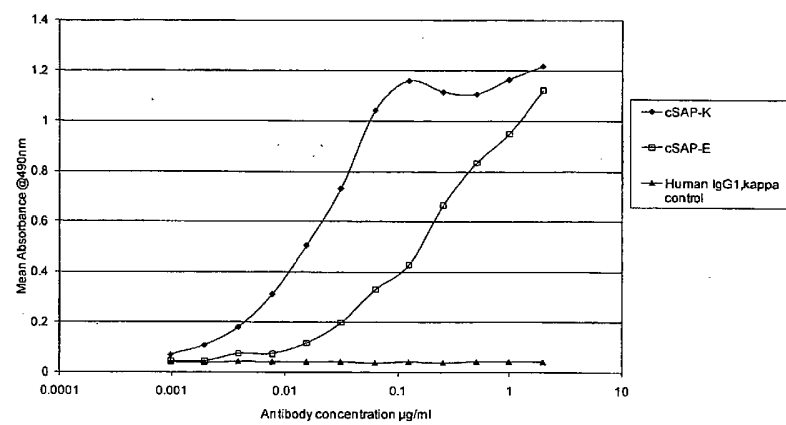


Figure 4

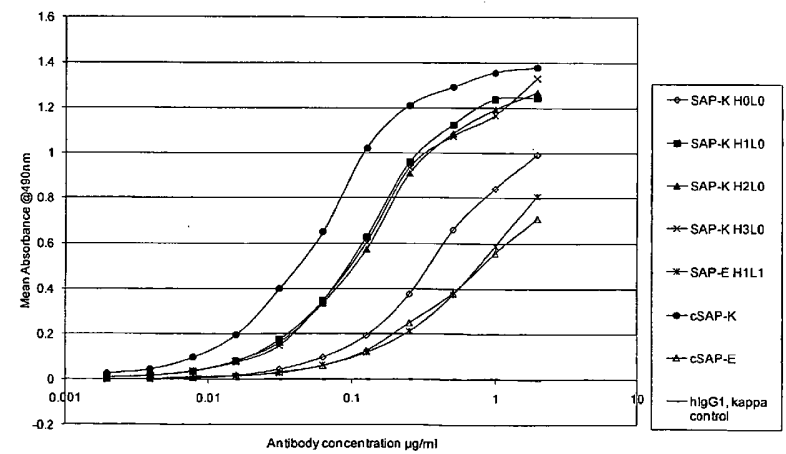


Figure 5

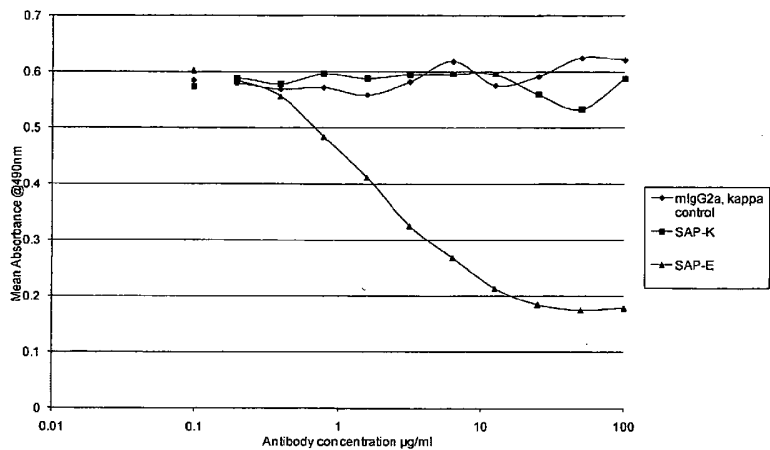


Figure 6

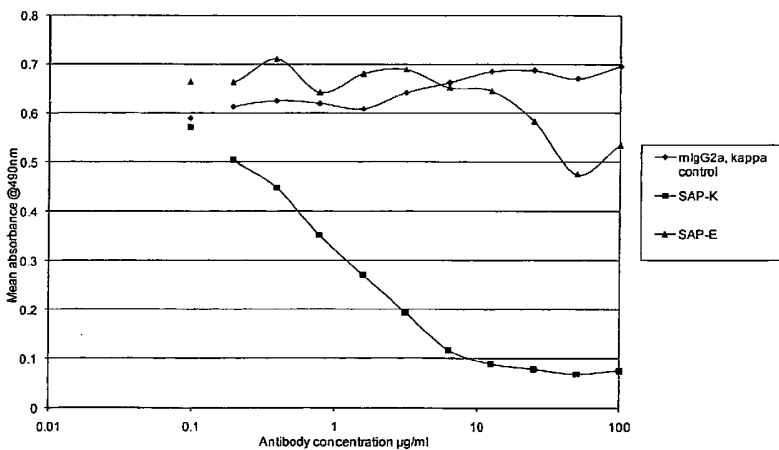


Figure 7

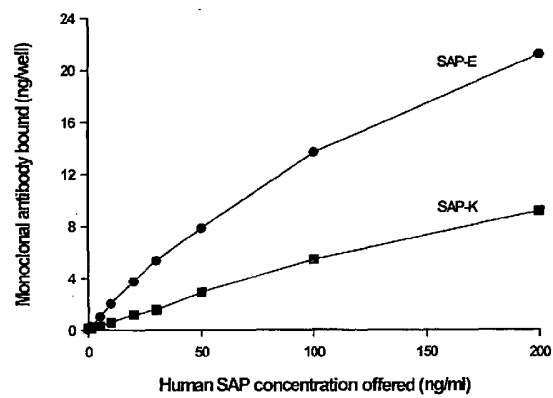
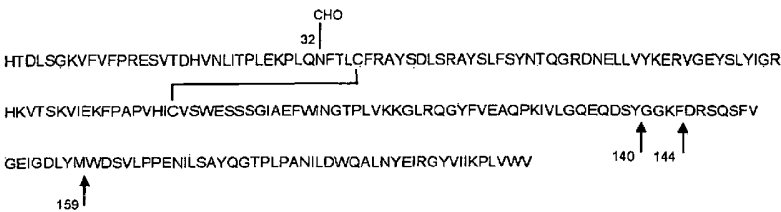
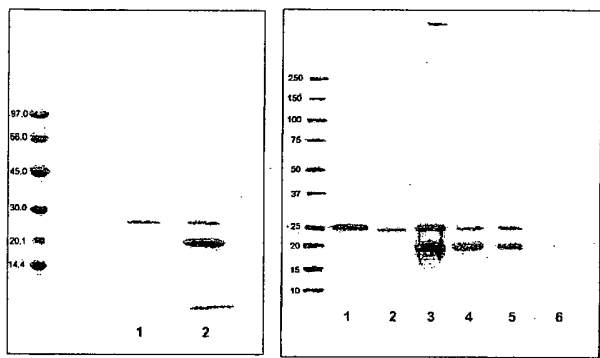


Figure 8

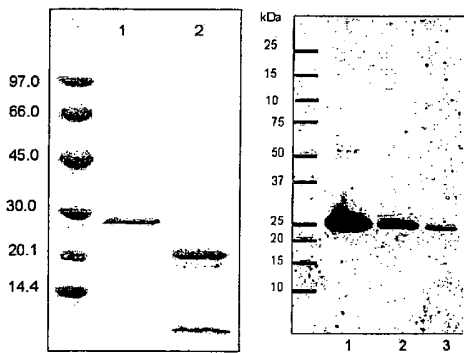
(A)



(B)



(C)



(D)

136 140 147
h EQDSYGGKFDRS
m EQDNYGGGFQRS

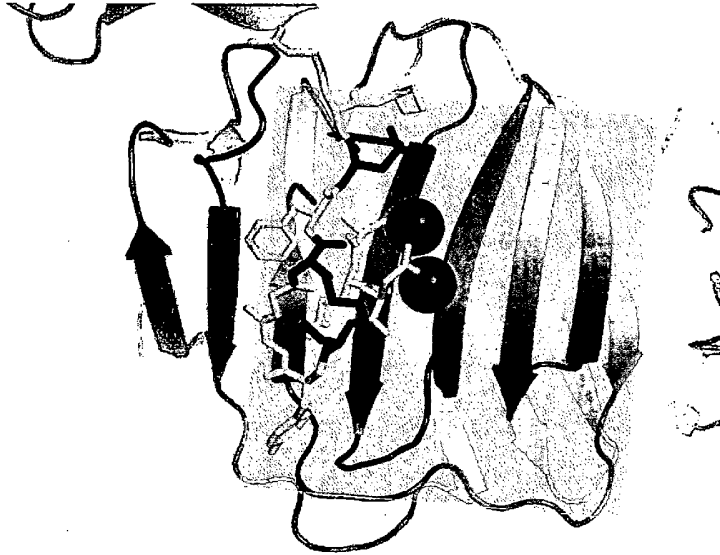
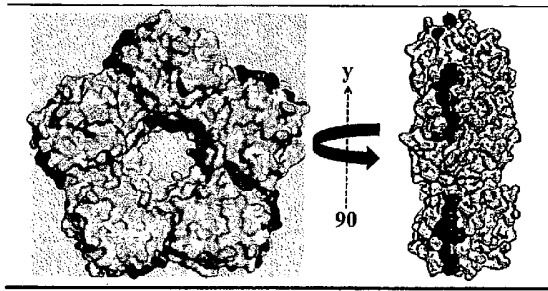


Figure 9

(A)



(B)

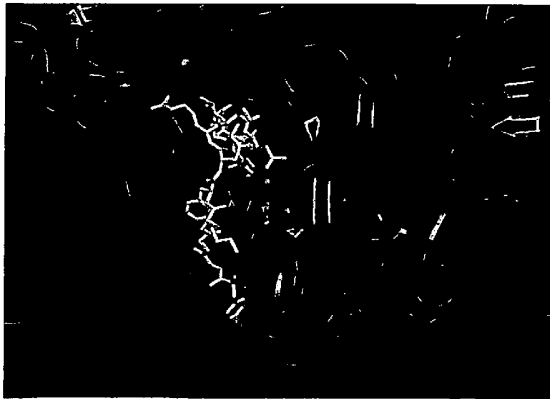


Figure 10

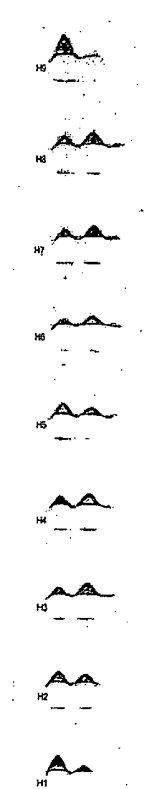


Figure 11

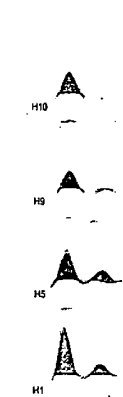
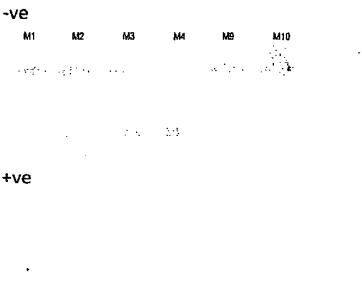
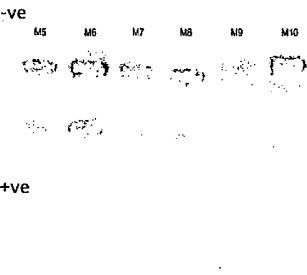


Figure 12

(A)



(B) Immunoblot probed with anti-mouse C3 at 1:5000



(C) Immunoblot probed with anti-mouse C3 at 1:10000

